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GROWTH AND NUTRIENT ACCUMULATION AS CONTROLLED BY OXYGEN SUPPLY TO PLANT ROOTS

H. T. Hopkins, A. W. Specht, and S. B. Hendricks

(WITH FOUR FIGURES)

Received December 1, 1949

Introduction

The possibility that oxygen may become so low in soil or other media as to limit plant growth has long been a matter of concern (**3**) (**4**). Numerous studies have adequately demonstrated that low oxygen partial pressures (**2**) (**3**) (**6**) (**12**) (**13**) (**14**) retard root growth. Extensive investigations on salt absorption and accumulation of excised roots and potato tissue discs (**8**) (**9**) (**10**) (**11**) (**20**) (**21**) (**22**) (**23**) (**24**) reveal the general dependence of respiration and salt accumulation on an aerobic metabolism. Vlamis and Davis (**25**) have noted the ability of rice and barley to accumulate ions under anaerobic conditions around the roots when the shoot was in air. Some work has been done which suggests that plants may make use of several systems for maintaining an oxidation level in the roots sufficient for the growth activities of the whole plant. Reference is made to the early work of Arnon (**1**) on the ammonium and nitrate nutrition of barley. Somewhat later, Shive, (**19**) and Gilbert and Shive (**7**) showed that soybean roots under anaerobic conditions consumed more nitrate nitrogen than when aerobic. Lundegardh's (**15**) work on intact wheat plants demonstrated the positive relationships between concentration of nitrate anions in the nutrient solution and respiration. Recently, Woodford and Gregory have shown that barley can grow as well under anaerobic conditions as aerobic, provided the nitrate anion concentration is increased several fold (**26**).

Despite these and other fundamental approaches to the subject, as well as many horticultural studies, the impression is widely held by soil workers that quantitative information is still greatly needed. Evaluation is particularly desired for effects of short periods of anoxia that might lead to prolonged physiological disarrangement or permit invasion of soil borne pathogens through injury. Knowledge is still lacking of the extent to which transport of some nutrient elements and water is limited by oxygen supply.

In the work described here attention was focused on the overall growth

response of several plants to low oxygen partial pressures ambient to the roots. Experimental conditions were established such that diffusion of oxygen to the root surfaces would not be a limiting factor. Nutrition and water supply were maintained at optimum levels for the plants studied. Changes in cell elongation, green weight, ion accumulation, and shifts in the internal nutritional status of the shoot were observed. Growth was followed in experiments of two-weeks' duration while periods of two or three days were used in studying uptake of radioactive isotopes. This approach was adopted in part to avoid the many questions that might arise in the bearing of studies on isolated roots on overall plant behavior.

Materials and methods

Large populations of *Nicotiana tobaccum* var. Maryland Mammoth and var. Connecticut broadleaf, *Lycopersicon esculentum* var. Marglobe, and *Soja max* var. Biloxi were grown from selected seed with an aerobic root environment. Uniform plants for treatment were selected from this population. All plants were grown in two-gallon urn-type glazed porcelain pots filled with eight-mesh quartz sand. Tobacco and tomato plants were transplanted from two-inch pots whereas soybeans were seeded directly into urns. Culture solutions which had been developed through long practice at this station were used for each plant; namely, a modified Shive's four-salt solution with added micro nutrients for tomato and soybean, and one used by McMurtrey (**17**) for tobacco. Nutrient solution was applied daily.

At a suitable time before the differential oxygen treatments the various pots selected for the tests were sealed with rubber caps which were bound down with masking tape. A very small opening in the cap around the plant stem allowed aerating gas to escape after passing upwards from the bottom of the pot. Perforated copper tubing placed on top of the sand and leading through the cap served as an efficient irrigation system after the pots were sealed. Mixed gases, consisting of water-saturated nitrogen and air combinations at various values over the range between 0.5% and 6.4% oxygen, were used at a flow rate of 10 liters per hour, which was adequate to flush twice each hour and to prevent back diffusion around the plant stem. Control plants received forced aeration, the air being filtered through packing waste after leaving the compressor. In order to facilitate distribution, a two-inch pot was inverted over the hole in the bottom of the urn through which the mixed gas was introduced. Channeling did not occur since the sand grains were large and quite uniform in size distribution. During the experimental runs, frequent analysis of the gas inside each pot was made as a check for leaks and to test the adequacy of the mixing technique. No difficulty was encountered.

Accumulation studies with P32 were carried out for tobacco and soybean whereas both K42 and P32, as supplied in an irradiated unit of KH_2PO_4 were used with tomato. The radioactive substances were made up in stock solution sufficient for an entire experimental period. The short half life, 12.4 hours,

of K42 dictated rapidity in all operations including final assay. Slower decay of P32 necessitated careful sanitation in disposal of wastes.

At the end of an experiment plants were minced in a food chopper and weighed samples were dried in a forced draft oven. After drying, the samples were digested with HNO_3 and $HClO_4$, diluted and filtered. Zirconium nitrate was then added to the approximately 25 cc volume in a 50 cc centrifuge tube in order to precipitate phosphate containing P32. After 15 minutes centrifuging, excess zirconium nitrate was removed by addition of inactive sodium phosphate. Potassium in the supernatant liquid was precipitated with sodium cobalti nitrite, the solution was cooled for one hour in a refrigerator, and then filtered on filter paper discs following the method of MacKenzie and Dean (**16**). Radioactivity was measured at once by use of a commercial bell type GM tube and associated scaling unit, after which the precipitate was dried and weighed. The specific activity was then calculated relative to that of potassium in the initial solution after necessary allowance for decay. Since the potassium cobalti nitrite precipitates were sometimes contaminated with adsorbed P32, all were allowed to stand for several weeks and the activity remeasured. A correction was then made for the initial counting. Total phosphorus and P32 relative activities were determined on an independent sample after the method of MacKenzie and Dean (**16**).

Gas mixtures of constant composition were obtained irrespective of variation in individual flow rates by means of the arrangement shown diagrammatically in figure 1.[1] A vessel B having a relatively great diameter was placed as indicated before the flow meter in the nitrogen line. A water connection led from B to the overflow A in the oxygen line. Change of the gas pressure in B changed the overflow height in A which thus shifted the air pressure at the air flow meter in the direction to maintain constant composition. Perfect control is only possible for an infinitely large diameter of B but practical control restricting variation of oxygen partial pressure to $PO_2 \times .05$ can be obtained with a reasonable diameter.

A total flow rate of the mixed gas was used such that five pots could be serviced from each source of supply. For a complete experimental run of two weeks, using a 5×5 setup, a total of 17 tanks (200 cu. ft.) of water-pumped nitrogen gas was required. Five of these were needed for the 0.5% series. Each tank of nitrogen gas was analyzed by means of a Pauling Oxygen meter before being put into the line. The advantage of this instrument was its accuracy in the low ranges, being especially built for the range below fourteen millimeters. The gas composition inside each pot was checked at least twice during the runs, using a portable Orsat apparatus. It was observed that the analytical error with the Orsat was in excess of the error involved in the mixing technique.

[1] Mr. R. A. Nelson, U. S. Geological Survey and formerly with this Division worked out the theory of the regulator.

Results

Growth Data

Response of tomato, tobacco, and soybean plants to various oxygen partial pressures and to forced aeration is shown in table I.

Experiment 1—Tomato plants, at about the onset of blooming, were subjected to oxygen levels of 0.5; 2.0; and 5.0% for a two-week period. Control plants received forced aeration. Three leaves, numbers 10, 11, and 12 (counting from base) were measured daily. The data in column 4 of table I refer to leaf number 12. The length includes the entire leaf structure, from its point of attachment at the stem to the extreme tip.

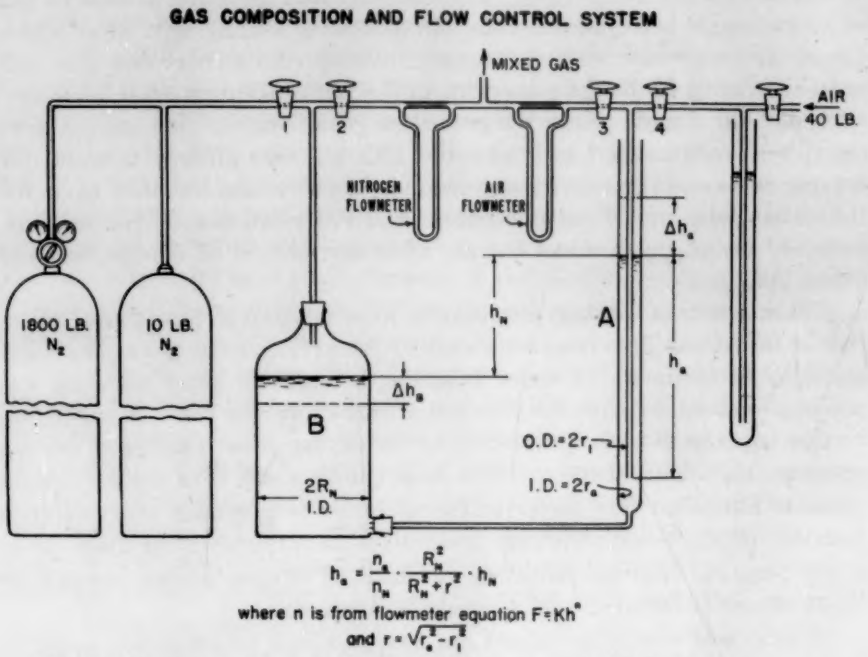

Fig. 1. Apparatus for maintaining constant gas composition.

The final green weights of tops and roots, as well as the total elongation of leaf and stem were significantly greater in the control plants than in any one of the other treatments. Furthermore, the growth of tomato plants was significantly reduced, at each oxygen level, relative to that at atmospheric pressure. The color of the leaves of the plants whose roots were exposed to oxygen pressures lower than that in air, was distinctly a paler green than that of the controls. Effects of low oxygen concentration were easily seen.

At harvest, the root system of the 0.5% series was distinctly smaller than the controls, and except for an outcrop of new roots above the level of the original root system, appeared brown and dirty. The outcrop of new roots produced in the 2% and 5% series was considerably more abundant than in

TABLE I

AVERAGE FINAL GREEN WEIGHTS, AVERAGE INCREASE IN LENGTH OF A SELECTED LEAF, AND THE SHOOT OF TOMATO, TOBACCO, AND SOYBEAN IN RELATION TO THE EXTERNAL OXYGEN PARTIAL PRESSURE IN THE ROOT ZONE.

Oxygen content %	Tomato				Tobacco (Maryland Mammoth)				Tobacco (Conn. Broadleaf)				Soybean			
	Green wt. gms.		Elong. mm.		Green wt. gms.		Elong. mm.		Green wt. gms.		Elong. mm.		Green wt. gms.		Elong. mm.	
	Tops	Roots	Leaf	Stem	Tops	Roots	Leaf	Stem	Tops	Roots	Leaf	Stem	Tops	Roots	Leaf	Stem
	42*	15*	0**	0**	168†	52*	0	0	85*	56*	0	0	36*	23*	0	0
0.5	62	11	48	95	134	40	98	70	193	36	142	311	55	23	321	413
1.5					338	81	165	189					62	36	317	403
2.0	80	18	151	172												
3.2					284	103	168	201					61	46	315	400
5.0	101	24	176	215					464	136	334	570				
6.4					297	89	148	191					60	42	325	394
Forced Aeration	123	30	204	261	338	96	222	217	497	134	373	580	68	43	301	372
LSD5%††	16	2.8	28	43	51	18	50	44	38	30	46	65	26	6.5	40	59
LSD1%	24	4.0	39	60	75	26	73	64	53	42	64	90	36	9.0	55	80

* These values are obtained from the initial population.
** Elongation values are given as differences between initial and final lengths.
† Dry weights indicated some growth had occurred; green weights of tops of other plants show same relationships as dry weights.
†† LSD denotes the least difference required for significance between any two means.

the 0.5% series but the original root systems in general were healthy looking. It was evident, however, that the original root system did not grow, in any except the control plants. Root-like outgrowths appeared on the stems of plants in the 0.5 and 2.0% series, below and above leaves four and five. No such outgrowths appeared on the stems of the 5% series or the forced aeration controls. Tomato root weights were more seriously affected than top weights by low oxygen partial pressures, as seen in table I, column 3.

The effect of low oxygen partial pressures on leaf and stem elongation was clearly evident 24 hours after the experiment started. The loss of green color in the leaves of the plants with roots in low oxygen was progressive, being strikingly evident by the fifth day in the 0.5% treatment, and by the ninth day in the 2% and 5% treatments.

Experiment 2—Maryland Mammoth tobacco plants can be maintained vegetative without supplementary light during the long days of summer. The growth data shown in table I are for such vegetative plants. Plants in an active stage of growth were subjected to gas with an oxygen content of 0.5, 1.5, 3.2 and 6.4% around the roots, with normal air used for controls. The leaf selected for daily elongation measurements was near the apex, being the fourth leaf down the stem axis, counting from an apical leaf having maximum dimensions of 20 by 40 millimeters.

Forced aeration of the rooting medium produced tobacco plants with tops having significantly higher green weights than plants whose roots were exposed to oxygen contents of 3.2, and 0.5%. The green weight of tops in the 1.5% treatment, and the green weight of roots in the 3.2% treatments were essentially equal to the controls, but significantly greater than any other treatment. An oxygen concentration of 0.5% adversely affected root growth. The color of tobacco leaves deteriorated only in the 0.5% treatment. The original roots of plants in the 0.5% series were essentially dead at the end of the experiment and a few new roots appeared above the original root system. The roots of the plants in the 1.5% and higher treatments remained healthy.

Elongation of the leaf was changed within 24 hours after treatments had started. The effect of low oxygen concentration on leaf elongation was significant in all oxygen levels lower than that in air, as shown in table I. The general appearance of the test plants, outside of the 0.5% treatment, was essentially like that of the controls. An oxygen content of 0.5% significantly reduced the elongation of the stem. The impact of low oxygen partial pressures on stem elongation of tobacco was not apparent until 48–72 hours after treatments had started.

Experiment 3—Connecticut broadleaf tobacco plants were used at first but later abandoned in favor of Maryland Mammoth since flowering introduced considerable variation. It was observed that wilting of the four basal leaves occurred within one hour after the start of the 0.5% oxygen treatment. Such wilting was at first intermittent, being most severe during days of high light intensity when greenhouse temperatures exceeded 90° F with the plants recovering at night. After five days wilting was permanent even though

relative humidity was high and the water supply to the roots entirely adequate. This effect is due to restricted transport of water since it takes place before a growth differential occurs.

EXPERIMENT 4—Soybean plants were maintained vegetative throughout their growth by illumination for one hour in the middle of the dark period. The growth data in the last four columns of table I reveal the remarkable tendency for soybean to maintain the processes of growth in all oxygen contents except 0.5%. At this level, the green weight of tops and roots was significantly altered. Leaf color was essentially the same in all except the 0.5% treatment. In this treatment the change to a light green developed slowly over the two-week experimental period.

TRANSFER OF RADIOACTIVE ISOTOPES AS CRITERIA OF OXYGEN EFFECTS

Transfer of P32 and K42 from nutrient solutions to the experimental plants was followed for short periods. Accumulation in all cases is expressed

TABLE II

ACCUMULATION OF RADIOPHOSPHORUS AND RADIOPOTASSIUM IN THE HARVESTED SHOOT OF YOUNG AND OLD MARGLOBE TOMATO PLANTS, AS INFLUENCED BY THE EXTERNAL OXYGEN PARTIAL PRESSURE IN THE ROOT ZONE.

OXYGEN CONTENT %	POTASSIUM		PHOSPHORUS	
	YOUNG PLANTS	OLD PLANTS	YOUNG PLANTS	OLD PLANTS
	C/sec./mgm K		C/sec./mgm P	
	($\times 10^{-6}$)	($\times 10^{-6}$)	($\times 10^{-6}$)	($\times 10^{-6}$)
0.5	9.5 ± 3	5.5 ± 2	248 ± 141	68 ± 0.7
2.0	21.8 ± 4	8.8 ± 0.2	294 ± 29	138 ± 8.6
5.0	39.5 ± 0.8	11.0 ± 0.2	358 ± 7	124 ± 0.7
FORCED AERATION	20.7 ± 1	14.7 ± 0.2	232 ± 2	223 ± 8.9
LSD5%*	5.1	10.6	355	32
LSD1%	9.3	19.2	648	58

* The least difference required for significance between any two means.

in arbitrary terms giving relative specific activities. Such values were obtained for the harvested shoots of tomato and tobacco plants grown in sand culture for two to three days under different oxygen partial pressures. Activity values for soybean shoots were expressed on the ash basis. In tomato, accumulation of P32 and K42 was measured in vegetative and in flowering plants. In tobacco, accumulation of P32 was observed at intervals over a 168 hour period following one application of P32 to the nutrient solution. After seven days these plants were harvested and the specific activity values were obtained on the shoot.

EXPERIMENT 1—A nutrient solution containing about one microcurie of P32 and K42 as KH_2PO_4 was added to each of a series of tomato plants, within one hour following imposition of the different oxygen tensions. About

TABLE III

ACCUMULATION OF P-32 IN MARYLAND MAMMOTH TOBACCO; ACTIVITY MEASURED AT TWO PLACES ON THE LIVING PLANT, AND ALSO ON THE HARVESTED SHOOT. GROUP B PLANTS HAD BEEN UNDER DIFFERENT OXYGEN TREATMENTS FOR ONE HOUR, WHILE GROUP A PLANTS HAD BEEN UNDER TREATMENT FOR SEVEN DAYS PRIOR TO ADDITION OF P-32 TO THE NUTRIENT SOLUTION.

Oxygen content %	Group A			Group B		
	Counts per min. per sq. millimeter leaf surface 24 hours after addition of P-32.		Harvested shoot; specific activity c/sec/mgm P. seven days after adding P-32	Counts per min. per sq. millimeter leaf surface 24 hours after addition of P-32.		Harvested shoot; specific activity c/sec/mgm P. seven days after adding P-32
	Living mid-leaf	Living tip-leaf		Living mid-leaf	Living tip-leaf	
	(1)	(2)	(3)	(4)	(5)	(6)
0.5	5 ± 5	37 ± 36	1.10 ± 0.86	28 ± 3	116 ± 6	2.55 ± 0.31
1.5	135 ± 12	160 ± 22	4.08 ± 0.36	77 ± 11	164 ± 27	3.61 ± 0.46
3.2	152 ± 4	206 ± 23	4.11 ± 0.17	77 ± 38	181 ± 27	3.85 ± 0.24
6.4	154 ± 20	206 ± 11	3.96 ± 0.32	121 ± 11	177 ± 4	3.67 ± 0.14
Forced Aeration	168 ± 27	231 ± 18	4.11 ± 0.38	118 ± 23	243 ± 23	4.06 ± 0.06
LSD5%*	48	104	1.24	68	46	0.72
LSD1%	70	152	1.81	100	67	1.06

* The least difference required for significance between any two means.

24 hours later a second application of the $K^*H_2P^*O_4$ was made. After about 65 hours the two groups of plants, young and old, were harvested and assayed. The specific activity values obtained are shown in table II. Accumulation of P* by young plants is obscured by a large variance in the particular experiment.

EXPERIMENT 2—Two groups of Maryland Mammoth tobacco plants were set up with the purpose of determining, among plants of the same age, whether time of exposure to low oxygen partial pressure in the root zone is an important factor in transport of P32. In Group A plants the particular treatment was maintained for seven days prior to addition of the tracer, while Group B plants from the same initial lot were allowed to grow normally during this time and were put under different oxygen tensions about one hour before adding the tracer.

TABLE IV

RELATIVE ACCUMULATION OF RADIOACTIVE PHOSPHORUS BY SOYBEAN AND CONNECTICUT BROADLEAF TOBACCO PLANTS, WHOSE ROOTS WERE EXPOSED TO VARIOUS OXYGEN PARTIAL PRESSURES.

OXYGEN CONTENT %	ACTIVITY, EXPRESSED AS COUNTS PER SECOND PER MILLIGRAM OF ASH	
	($\times 10^{-6}$) SOYBEAN	($\times 10^{-6}$) TOBACCO (CONN. BROADLEAF)
0.5	403 ± 48	201 ± 22
1.5	687 ± 38	
3.2	769 ± 57	
5.0		451 ± 34
6.4	799 ± 38	
Forced aeration	817 ± 13	327 ± 32
LSD5%*	162	90
LSD1%	236	136

* Least difference required for significance between any two means.

Using a portable Geiger-Müller counter with an especially shielded probe, it was possible to measure, at short intervals following addition of P32, the transport of this tracer into previously marked leaves. The leaves were at the same position on the stem axis of each plant and are referred to henceforth as the mid-leaf and the tip-leaf. The results obtained are shown in table III.

EXPERIMENT 3—Biloxi soybean plants, kept vegetative by illumination for one hour in the middle of the dark period, were put under different oxygen tensions about one hour before adding P32. A second addition of P32 was made about 24 hours later. Within 65 hours the soybean shoots were harvested, dried, and ground. Samples were ashed and the activity values per milligram of ash obtained. These data are shown in table IV together with results for Connecticut broadleaf tobacco.

TABLE V

CHEMICAL COMPOSITION ON A DRY WEIGHT BASIS AND TOTAL INTENSITY OF INTERNAL NUTRITION OF TOMATO, TOBACCO, AND SOYBEAN TOPS, AS A FUNCTION OF OXYGEN PARTIAL PRESSURE IN THE ROOT ZONE.

Oxygen content %	B *p.p.m.*	Mn *p.p.m.*	Fe *p.p.m.*	Al *p.p.m.*	Cu *p.p.m.*	Na *p.p.m.*	K *p.p.m.*	Ca *p.p.m.*	Mg *p.p.m.*	P *p.p.m.*	Total Intensity Internal Nutrition *p.p.m.*
					Elemental composition of tomato tops						
0.5	14	79	71	26	8.0	673	30300	24475	10195	8158	73999
2.0	13	83	69	22	11.4	581	33600	24775	10360	8540	78054
5.0	11	77	91	26	7.8	539	36000	26550	10040	8765	82107
21.0	10	94	141	22	10.7	467	40200	26350	10435	9880	87610
LSD5%*	2.0	15	24	9.0	3.5	42	3234	2358	1612	965	
					Elemental composition of tobacco tops (Maryland Mammoth)						
0.5	41	73	125	45	27	702	22500	32080	4264	3967	63824
1.5	43	82	236	54	34	612	22700	38750	5152	5580	73243
3.2	46	77	116	42	31	458	24600	39166	5628	5238	75402
6.4	43	76	131	42	23	477	26800	35330	5185	4831	72938
21.0	44	90	154	45	18	502	28700	41420	5790	5733	82496
LSD5%*	6.4	22	52	8.8	9.0	110	2329	7366	1173	857	
					Elemental composition of soybean tops (p.p.m.)						
0.5	47	85	49	32	7.6	415	18200	12608	6180	4120	41744
1.5	53	81	50	30	7.1	348	21900	14820	6130	5940	49359
3.2	61	80	61	30	6.9	432	22300	15825	6400	6300	51496
6.4	57	97	53	28	7.0	308	22500	15500	6290	7370	52210
21.0	54	81	53	30	5.0	249	24400	15100	6840	7430	54242
LSD5%*	8.1	16	6.5	4.6	1.2	92	1991	1676	1581	608	

* Least difference required for significance between any two means.

Internal nutritional status

Samples of the harvested shoot of each plant were analyzed from the two-weeks' experiment from which growth data in table I were obtained. A spectrographic assay was made using complete burning in a D.C. arc with comparison standard technique (**5**) for boron, manganese, iron, aluminum, copper, sodium, calcium, magnesium and phosphorus. Potassium was determined with a flame photometer (**18**). Results are shown in table V.

Discussion

Root growth of tomato, tobacco, and soybean is stopped by reducing the oxygen content of the surrounding gas to 0.5% but top growth continues. Data in table I show a small but significant decrease in root weight for the first two species over a period of two weeks, as might be expected if respiration is continuing on reserves in the roots rather than on materials principally translocated from the shoot. The weight of the tops increased by about 50% or more in each case, the actual green weight value for Maryland Mammoth tobacco being reduced by drying of the lower leaves with use of 0.5% oxygen around the roots. The rate of top growth at the 0.5% oxygen level relative to controls having forced aeration was markedly greater for soybean than for tomato and tobacco. In fact, leaf and stem elongation were somewhat enhanced, though not significantly. This, and the healthy condition of roots as well as their constant weight, indicated transport of elaborated materials from the shoot to the root in soybean even in 0.5% oxygen.

Change in top composition as a function of oxygen pressure in the root zone, average values for which are recorded in table V, reflects the effect of growth and any accumulation or losses. Increase in total weight of tops and roots for tomato and soybean for 0.5% oxygen around the roots is about 30% of the initial weight. If the shoot composition with regard to P and K is taken as approximately representative of the whole plant then in the absence of accumulation the amount of each element listed should be reduced by about 23%, i.e. $\frac{130-100}{130} \times 100 = \%$. This requirement is met for K and P in tomato as well as K in soybean, but the considerable values of LSD5% both for growth and analysis should be noted. The effect is paralleled by potassium and phosphorus in Maryland Mammoth tobacco, but the irregularity of the growth at 1.5% oxygen content of the gas in the root zone in the sequence of values leads to some uncertainty. At the lowest oxygen levels, the accumulation of phosphorus and potassium is reduced sharply toward zero.

Use of radioactive phosphorus and potassium indicated that these elements were transported from the nutrient solutions to the shoots in all treatments. It is unlikely that activity values are seriously affected by exchange between the element in the root and the nutrient solution, but rather that they reflect true accumulation for the particular time intervals under ob-

servation. If this is the case they serve as far more accurate measures of accumulations as functions of oxygen pressure in the root zone than do the overall compositional responses since measurements can be made on plants used to assess growth.

Accumulations of K42 and P32 by old tomato plants for 0.5% oxygen content of the gas in the root zone, listed in table II, are about ⅓ and ¼ as great respectively as the values for forced aeration. Such accumulations are within the limits of overall change in plant composition as discussed above.

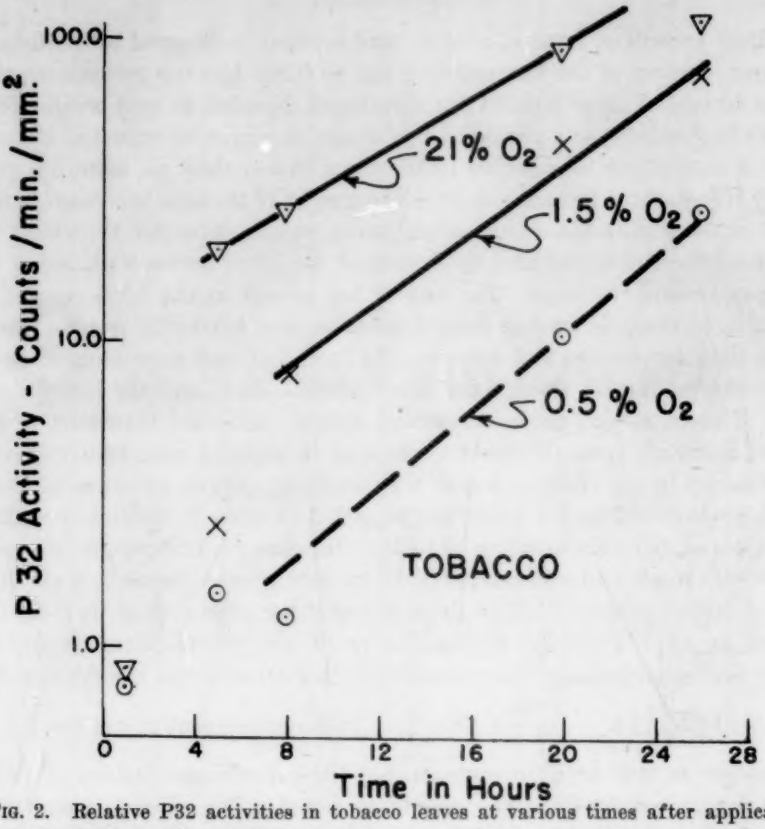

FIG. 2. Relative P32 activities in tobacco leaves at various times after application to nutrient solution. All activities are zero at zero time.

Accumulation of P32 in the relatively rapidly growing tip-leaf of tobacco is shown in table III. Change of activity is solely due to exchange between the phosphorus initially present in the area used for measurement and that coming into the growing leaf. The tip-leaf, because of its more rapid growth, shows greater activity than does the mid-leaf.

Change in relative activity of P32 in tobacco leaves was followed as a function of time in all cases. Results in strictly typical cases are shown in figure 2. It is evident in these examples, and true in all others, that equilibra-

tion is complete in about thirty hours. The rate of increase in P32 activity is similar in all cases irrespective of oxygen partial pressures over a considerable period where the activity follows an expression of the form,

$$\text{activity} = ae^{kt}$$

t, being the time and a and k constants.

It follows that,

$$\frac{d(\text{activity})}{dt} = \text{rate} = ake^{kt} = k\,\text{activity}$$

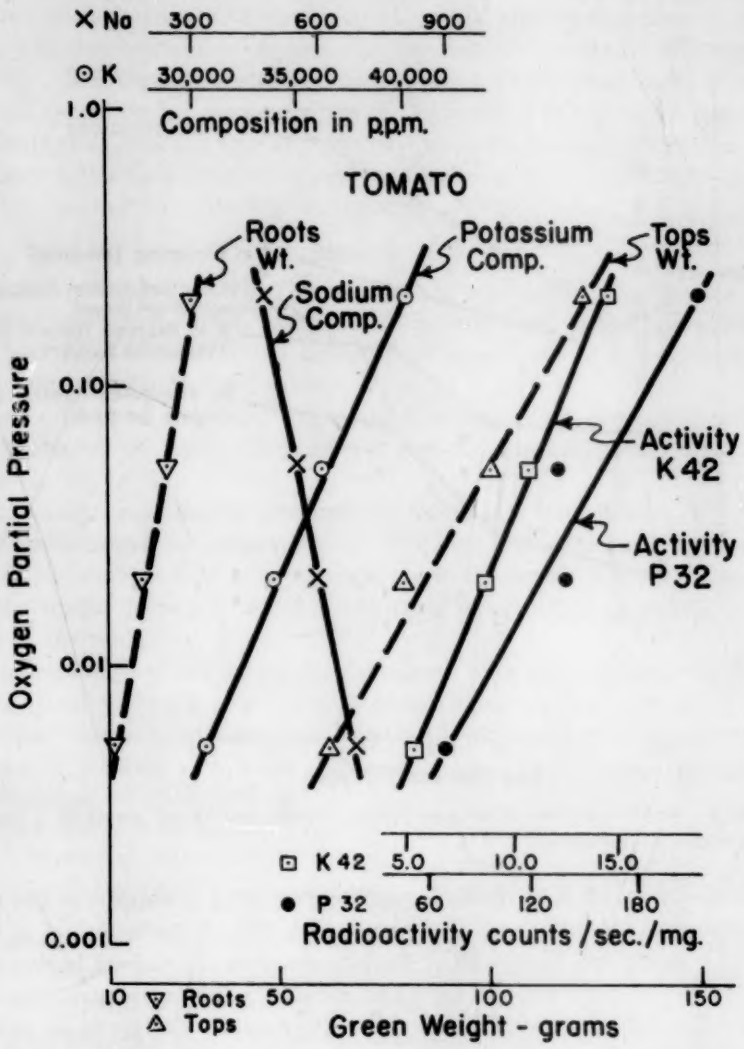

FIG. 3. Growth response of tomato at various oxygen partial pressures around the roots.

or log (rate) α time. The rate of transfer from the root to the shoot is thus independent of oxygen content of the gas ambient to the root.

Response of tomato to variation of oxygen content of the gas around the root is graphically presented in figure 3. Some values in the tables are aberrant, as well might occur for experiments of this type. A remarkably great amount of the data follows the form,

$$\text{response } \alpha \log pO_2$$

over the entire range from 0.5% to 21% oxygen. Inspection of the data for soybean in the several tables, on the other hand, shows that the response is a function of pO_2 only below 3.2% and possibly even lower values.

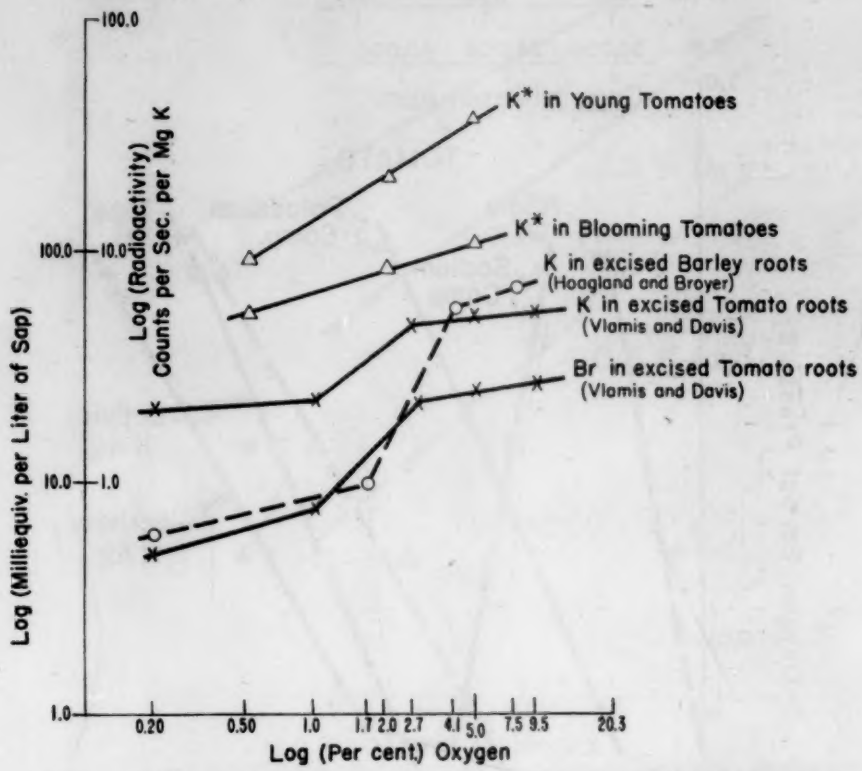

FIG. 4. Relative uptake of various ions by tomato and barley plants as a function of oxygen partial pressure.

Accumulation of K42 in its dependence on oxygen supply to the roots of tomato plants is contrasted in figure 4 with that of excised roots as measured by VLAMIS and DAVIS (**25**). Results obtained for excised barley roots by HOAGLAND and BROYER (**11**) are also included. The responses appear to be somewhat different, particularly when the overall data given in figure 2 are considered. An attached root can, of course, receive energy-supplying compounds by translocation from the shoot while the detached root with high

carbohydrate reserve depends necessarily upon synthesis in place. The evidence that translocation is unimpaired by limited oxygen supply is important in this respect.

Elemental compositions as listed in table V indicate that amounts of the major nutrient elements K, Ca, Mg, and P closely parallel growth response. Magnesium in tomato is an exception, remaining constant as the oxygen content of the gas around the roots is decreased. Manganese and iron in tomato also decrease monotonously with limited oxygen supply. This elemental behavior possibly expresses the "chlorosis" response of tomato in the experiments.

The trace elements, with the exception of Mn and Fe in tomato as noted above, remain constant or increase as the oxygen supply to the roots is lowered. The increase is striking for sodium. Transfer of these elements from the relatively low concentration of the medium to the higher values in the plants thus does not appear to depend upon an aerobic mechanism. Injury cannot be a factor since it would tend to bring about uniformity of composition; i.e., marked reduction in the root.

Summary

Growth response, nutrient accumulation, and nutrient transport were measured for tomato (var. Marglobe), soybean (var. Biloxi) and tobacco (var. Maryland Mammoth and Connecticut broadleaf) in their dependence on oxygen supply in the root zones.

Root growth of all plants was stopped at an oxygen content of 0.5% in the gas around the roots. Top growth and ion accumulation continued at this level.

In tomato, response was proportional to log pO_2 from 0.5% to 21% oxygen for growth and ion accumulation. The log (transport rate) was a function of time independent of oxygen supply to the roots. This indicates that rate of transfer from the root to the shoot is independent of aerobic mechanisms in the roots.

Accumulation of major nutrient elements with the exception of Mg for tomato parallels top growth in dependence upon oxygen supply to roots. Minor nutrient elements with the exception of Mn and Fe for tomato remain constant or increase with lowered oxygen levels. The increase of sodium is most striking.

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COMPOSITION OF DEVELOPING PEANUT SEED[1]

T. A. PICKETT

(WITH EIGHT FIGURES)

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In a review of plant fats in relation to environment and evolution, MCNAIR (5) noted that several factors influence fat production; stage of development, carbohydrate percentage, vegetative vigor, heredity, and environment. IVANOW (4) showed that the percentage of oil increased in ripening flaxseed as the percentage of sugars decreased. In general, studies on the composition of maturing oil seeds have usually dealt only with the quantitative and qualitative aspects of the crude lipid fraction and in some cases with the soluble sugars. MARKLEY (6) pointed out, however, that moisture content, weight of seed, and percentage of other constituents in addition to histological and anatomical data are also necessary for proper interpretation of the synthesis of fatty acids in the growing plant.

PATEL and SESHADRI (7) tagged flowers and reported that the bunch peanut of the Spanish type locally known in India as Gudiyattum required 95 days from planting or 60 days after flowering to produce mature nuts. Although tagging the flowers was of distinct value in evaluating the age of the nuts, the authors indicated that the seeds from flowers which opened on the same day did not develop at the same rate. The gynophores from the flowers near the soil entered the earth and produced seeds earlier than those more distant. They noted that the percentage of oil on the dry basis increased very slowly at the beginning and ending of the developmental period. Oil was synthesized rapidly between the 32nd and 46th day after flowering. GARNER, *et al.* (3) found that under the conditions of their studies climatic variations had more effect on size of seed and on oil content than did differences in the soils. GALLUP and STATEN (2) compared peanut fruits taken at a stage of growth about five weeks prior to normal harvest with those obtained at normal harvest. On a dry weight basis, the percentage of ash, protein, and crude fiber showed little or no change, while the ether extract fraction increased from 31.28% to 36.11%.

The fruiting habit of the peanut is peculiar; the flowers develop above the ground, but after fertilization of the ovule the stalk of the ovary lengthens. The elongating gynophore, popularly known as a "peg", pushes the ovary into the soil where it develops into shell and seed. Since blossoming is progressive over a period of several weeks, difficulty in determining the exact stage of development of the fruits by the planting date alone is quite evident. It is generally believed that in the Piedmont area of Georgia, Spanish type

[1] Paper No. 187, Journal Series, Georgia Agricultural Experiment Station, Experiment, Georgia.

peanuts require a minimum of 110–120 days from planting for maturity and the Virginia type about 130–140 days. A simple objective method for the determination of maturity is highly desirable.

This study involving two seasons' results was undertaken specifically to determine the composition of the developing seed and length of developmental period. The composition of the developing shell was also studied, but only for one season.

Materials

During the first season, samples of peanuts from uniformly well developed and productive small seeded Virginia Bunch plants grown at Experiment, Georgia, were removed from the field September 25, 1947, and at intervals thereafter the last sample being taken on November 25. They were transferred immediately to the laboratory where they were shelled and the seeds were divided into one or more groups, as described in table I. The very

TABLE I

CHARACTERISTICS OF VIRGINIA BUNCH PEANUTS HARVESTED AT VARIOUS STAGES OF DEVELOPMENT, 1947

STAGE OF GROWTH	COLOR OF TESTA	APPEARANCE OF INSIDE OF SHELL	ESTIMATED TIME REQUIRED FOR MATURITY
			Days
Very Immature			
"Second Setting"	White	White, Thick and Fleshy	35
Immature	White	White, Fleshy	28
Partially Mature	Slightly Pink	White, Papery	14
Mature	Pink	Brown and White	.....
Over-Mature	Pinkish-Brown	Predominantly Brown	.....

immature group, harvested late in the season, was considered to be the result of a late flush of blooming.

Virginia Bunch strain (Georgia Station Introduction 67–14) was planted May 10, 1948, in rather low and fertile soil, and subjected to the usual cultural practices. On July 19, 70 days after planting, between 3000–4000 gynophores, just entering the soil, were tagged at random throughout a one-half acre plot. It was estimated that the time between the appearance of the flowers and the entrance of the gynophores into the soil was about 14 days. After tagging, soil was gently placed over the tip of the gynophore. During the 1948 season, samples were obtained by the following procedure: Samples were taken at random on the 9th, 18th, and 28th day, and at weekly intervals thereafter until the 70th day after tagging. The sample taken on the 70th day after tagging had the appearance of being definitely over-mature, as shown by the color of testa and shell. With the exception of the ninth day sampling, where only a few pods were removed, a sample consisted of 600–700 fruit and diminished to 200–300 fruit as the peanuts matured. The peanuts were washed and drained in a basket centrifuge which removed

the major portion of the surface water. After shelling, the obviously abnormal seeds and shells were discarded and the rest usually were divided into two groups each of seeds and shells.

From the 18th through the 49th day, samples of seeds were divided into two groups on a weight basis; for example, on the third sampling date the seeds were divided into those weighing more than or less than 500 mg. The 56th, 63rd, and 70th day samples of both the seeds and shells were subdivided into two groups each, depending upon the color of the testae and of the inside of the shells.

Methods of analysis

Counted numbers of seeds and samples of shells were weighed and dried to constant weight at 65° C in a forced draft oven. The materials were then weighed and ground. Moisture, crude fat, and crude protein (nitrogen × 5.5 for seed and nitrogen × 6.25 for shells) were determined by the official methods of analysis (**1**).

The Shaffer-Somogyi reagent (**8**) was used in the determination of reducing and total sugars, sucrose being inverted with invertase. Starch was determined on the 80% alcohol insoluble residue by incubation with diastase and subsequent acid hydrolysis. The acid hydrolyzable fraction, commonly called hemicellulose, was obtained on the starch residue from the second season samples by using a 3% HCl hydrolysis for three hours in a boiling water bath. Reducing sugars, sucrose, and the hemicellulose fraction are reported in terms of dextrose and starch as dextrose × 0.9.

Weather, as well as the strain of peanuts and method of determining their physiological age, differed for the two seasons; however, data on the composition of the ripening seeds were comparable. During the first season, this study covered only the later stages of growth and age of samples was judged solely by appearance. During the 1948 trial, samples were obtained over a longer period and age was based on date of entrance of gynophores into the soil. These samples were further divided on a weight and appearance basis.

Results

1947 Season.—The data obtained during the first season on Virginia Bunch seed are summarized in table II. These results expressed in both percentage and mg. per seed were compiled from data obtained on groups of two or more samples harvested at six different dates.

As maturity approached, the water content of the seeds decreased from 62% to the usual (30–40%) range of fresh mature seeds. It appeared that there was a difference of about two weeks in the physiological age of the partially mature and mature groups. The mature group had increased only 4% in fat over those partially mature, which is in agreement with the results obtained by Patel and Seshadri (**7**). In contrast on a weight per seed basis the increase in fat was 200 to 270 mg., or 35% increase in fat in the two-week period immediately preceding maturity, due mostly to the large increase in dry weight per seed. The total sugars and starch decreased from 30.3 to

TABLE II

COMPOSITION OF VIRGINIA BUNCH SEED—1947.

MATURITY OF SEED	COLOR OF TESTA	MOISTURE	DRY BASIS							PER SEED BASIS					
			CRUDE FAT	CRUDE PROTEIN	REDUCING SUGARS	SUCROSE	STARCH	TOTAL SUGARS AND STARCH	AVG. WT. DRY BASIS	CRUDE FAT	CRUDE PROTEIN	REDUCING SUGARS	SUCROSE	STARCH	TOTAL SUGARS AND STARCH
		%	%	%	%	%	%	%	mg.	mg.	mg.	mg.	mg.	mg.	mg.
Very immature	White	61.8	27.3	18.6	3.6	3.2	23.5	30.3	275.	75.	51.	10.	9.	65.	84.
Immature	White	52.2	46.0	25.4	1.2	2.4	8.1	11.7	245.	113.	62.	3.	6.	20.	29.
Partially mature	Slightly Pink	42.3	50.9	26.5	0.7	1.2	5.1	7.0	393.	200.	104.	3.	5.	20.	28.
Mature	Pink	33.1	52.9	26.1	0.4	1.5	3.5	5.4	510.	270.	133.	2.	7.	18.	27.
Over mature	Pinkish brown	30.5	53.7	25.4	0.4	2.0	3.0	5.4	522.	280.	132.	2.	11.	16.	29.

5.4% as maturity approached. Sucrose increased slightly when seeds were over-mature. On a mg. per seed basis, there was a marked decrease in carbohydrates between the very immature and the immature groups. From the latter stage until maturity, however, the total sugars and starch remained constant.

The ratios of protein-carbohydrate, fat-carbohydrate, and fat-protein increased as maturity approached (table III). In the case of protein-carbohydrate ratios, protein remained relatively constant, and the increase was caused by the decrease in carbohydrates. The increase in the fat-carbohydrate ratio was due to a rise in fat while the carbohydrates decreased. The rise in the fat-protein ratio was due to the fat increasing as protein remained constant.

TABLE III

FAT, PROTEIN, AND CARBOHYDRATE RATIOS OF VIRGINIA BUNCH SEED—1947.

MATURITY OF SEED	CRUDE PROTEIN / TOTAL SUGARS AND STARCH	CRUDE FAT / TOTAL SUGARS AND STARCH	CRUDE FAT / CRUDE PROTEIN
Very immature	0.6	0.9	1.5
Immature	2.2	4.0	1.8
Partially mature	3.8	7.2	1.9
Mature	4.9	9.8	2.0
Over mature	4.7	9.9	2.1

1948 SEASON.—Although the gynophores were selected rather carefully at tagging, considerable variation in appearance of the Virginia Bunch seeds at each sampling date was noted. The samples were divided first by weight and toward the end of the ripening period also by color of the testae and the inside of the shells. A difference of as much as seven days in apparent physiological age was noted between samples harvested on the same day (table IV).

The data (table IV) obtained from the samples were calculated to weighted mean values on a weight per seed, percentage fresh weight, and percentage dry weight basis for the whole sample at each harvest date (figures 1–8). Although mean values are given for the 70th day sample, both subsamples were overmature in appearance and the variation in composition, size, and number of seed was very large. The smaller sample had decidedly abnormal characteristics and the carbohydrate fractions were not determined.

While the seeds attained a peak in fresh weight on the 49th day after tagging, the dry weight continued to increase (fig. 1).

It is known that the shells of cured as well as fresh mature peanuts

TABLE IV

COMPOSITION OF VIRGINIA BUNCH SEED—1948.

DAYS AFTER TAGGING	SEED* USED	MOISTURE FRESH BASIS	AVERAGE WEIGHT OF SEED DRY BASIS	CRUDE FAT DRY BASIS	CRUDE PROTEIN DRY BASIS	REDUCING SUGARS DRY BASIS	SUCROSE DRY BASIS	STARCH DRY BASIS	HEMICELLULOSE FRACTION DRY BASIS
No.	*No.*	*%*	*mg.*	*%*	*%*	*%*	*%*	*%*	*%*
18	300	81.5	17.	8.0	23.9	1.4	8.2	24.8	3.7
18	38	79.5	56.	17.1	21.7	0.5	8.4	25.7	2.9
28	260	76.3	70.	24.3	21.7	0.5	6.0	25.4	2.8
28	140	69.1	190.	32.5	21.1	0.3	5.7	24.7	2.7
35	35	68.6	132.	32.1	22.3	0.4	6.2	22.4	2.5
35	41	62.0	265.	39.3	21.5	0.2	5.0	17.1	2.8
42	80	57.4	226.	41.3	24.1	0.2	4.8	13.2	2.7
42	70	52.9	382.	45.7	23.5	0.1	4.4	12.0	2.3
49	66	46.2	306.	48.9	25.1	0.1	2.9	7.6	2.6
49	56	46.2	417.	50.5	24.5	0.1	2.9	7.8	3.0
56	84	48.1	406.	48.5	25.3	0.1	3.2	8.4	3.2
56	200	37.1	504.	51.8	25.9	0.1	2.2	5.9	2.7
63	53	43.2	452.	50.1	25.7	0.1	2.6	6.0	2.7
63	100	33.5	517.	51.6	25.8	0.1	3.0	4.5	3.1
70	100	33.2	573.	49.5	26.2	0.1	4.3	4.1	3.0
70	26	34.6	428.	48.8	26.2				

* In addition to those used for study of the carbohydrate fractions.

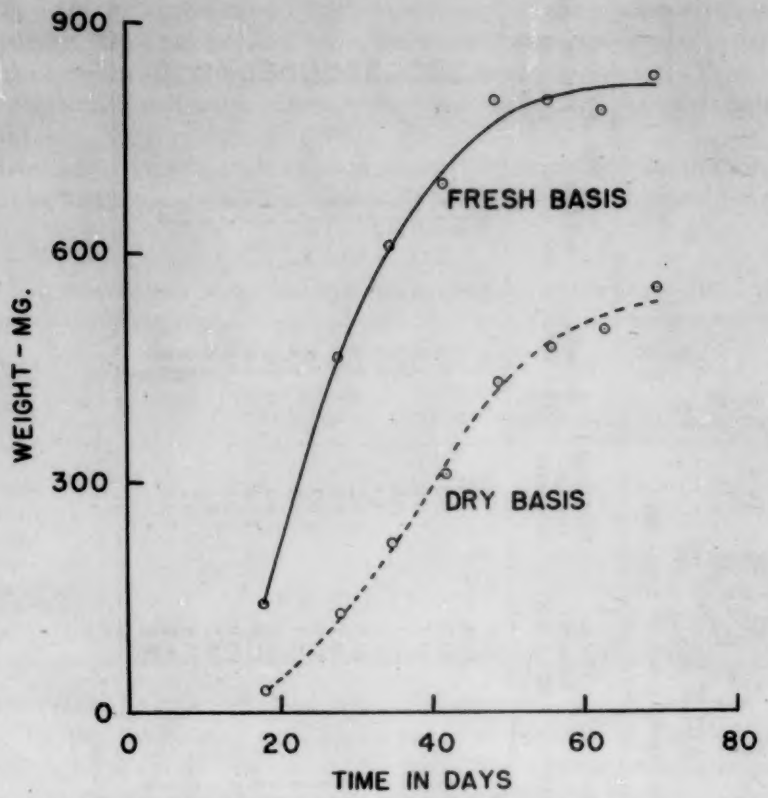

FIG. 1. Average weight of individual Virginia Bunch seed, 1948.

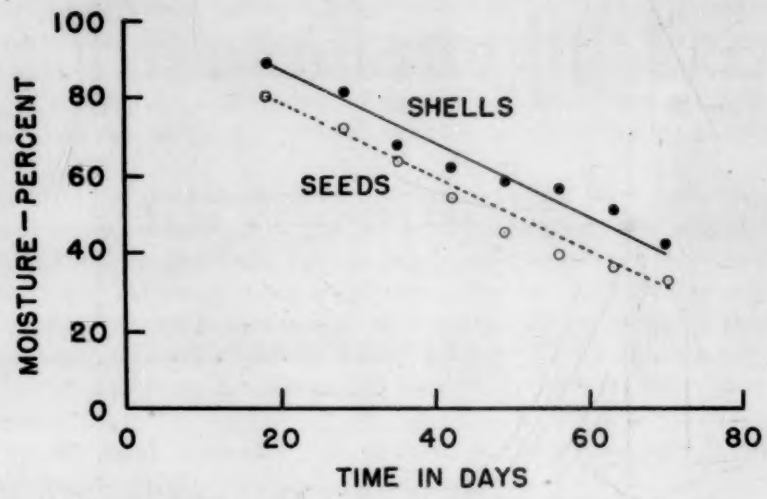

FIG. 2. Percentage of moisture in Virginia Bunch seed and shells, 1948.

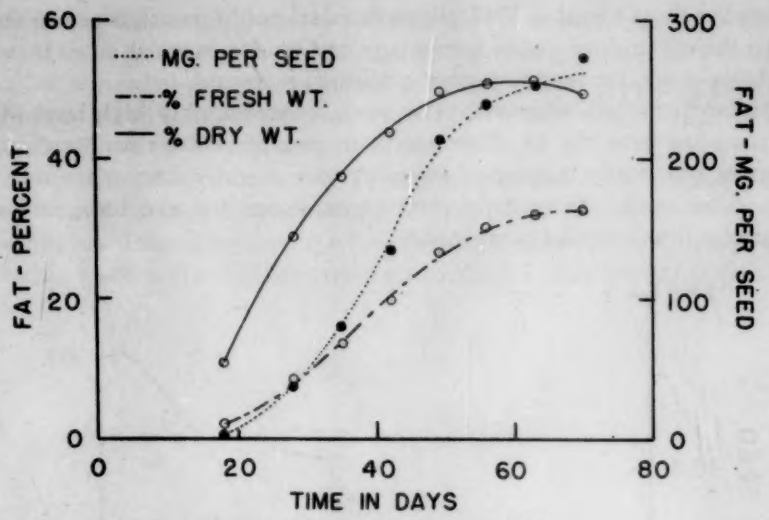

FIG. 3. Crude fat content of Virginia Bunch seed, 1948.

(moisture content in equilibrium between shell and seed) have a higher moisture content than the seed. A similar relationship was obtained on seeds and shells of all samples harvested from the 18th to the 70th day after tagging as shown by the parallel curves obtained from the moisture data (fig. 2).

Fat data expressed in percentage of fresh and dry weight, as well as per seed basis, are given in figure 3. Although the differences were not as pro-

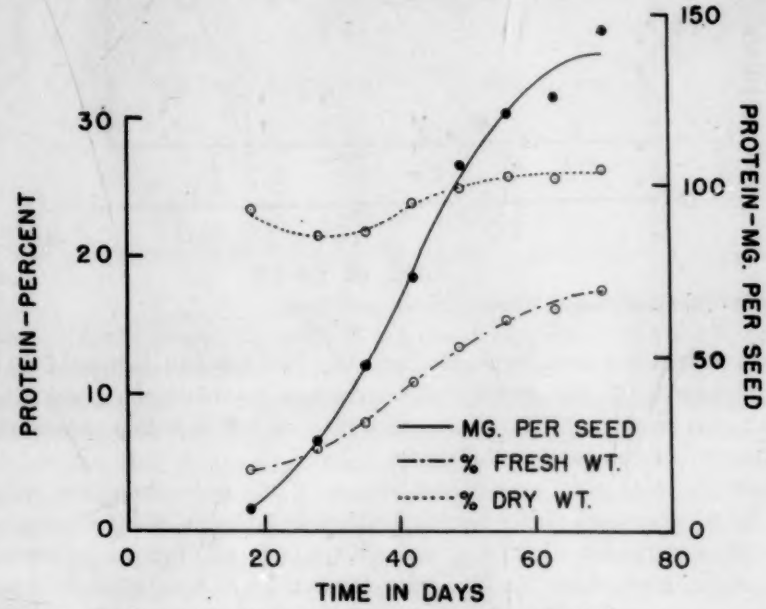

FIG. 4. Crude protein content of Virginia Bunch seed, 1948.

nounced as those found in 1947, the same relationship was obtained as shown by the change in slope of the percentage dry weight curve at 49 days, while on a basis of mg. per seed the curve continued to ascend.

Although protein appeared to be at an unaccountably high level at the first sampling date (fig. 4), this constituent on a percentage dry weight basis remained practically constant, increasing only slightly during the development of the seeds. In contrast, protein on a mg. per seed basis increased rather steadily as the seeds ripened.

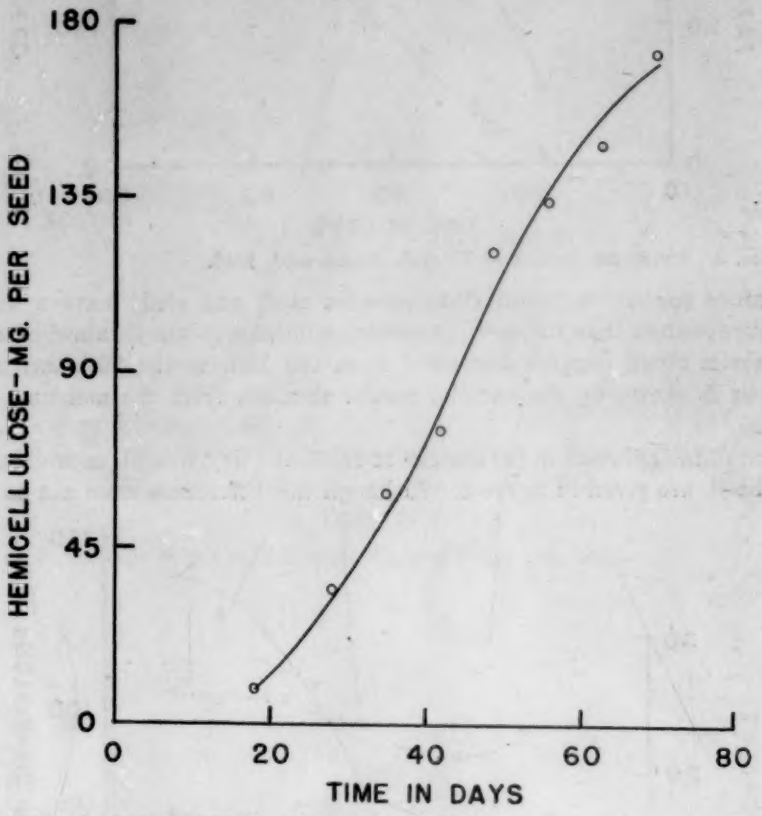

FIG. 5. Hemicellulose in Virginia Bunch seed, 1948.

On a weight per seed basis, the hemicellulose fraction increased as the seeds developed (fig. 5). Some of the variations shown on the graph might be due to the method of determining this fraction which is less precise than other determinations used in this study.

Generally, both total sugars and starch on the percentage dry weight basis (fig. 6) decreased as the peanut developed; however, the percentage of total sugars increased slightly at maturity due to an increase in sucrose. This increase, also evident in 1947, may account for the slightly sweet taste of fresh mature seed. With the exception of very immature seed, reducing

sugars during the development were found only in traces. Although starch decreased on a percentage basis throughout the whole period, this decrease leveled off somewhat towards maturity so that the total soluble sugars and starch approached a constant value near maturity somewhat similar to that obtained on Virginia Bunch seeds in 1947.

On a weight per seed basis (fig. 7) starch increased in the earlier stages, then decreased from mid-development to over-maturity. In general, total sugars increased throughout most of the development period. Although only one sample was examined at the over-mature stage, a high level of sugars was noted.

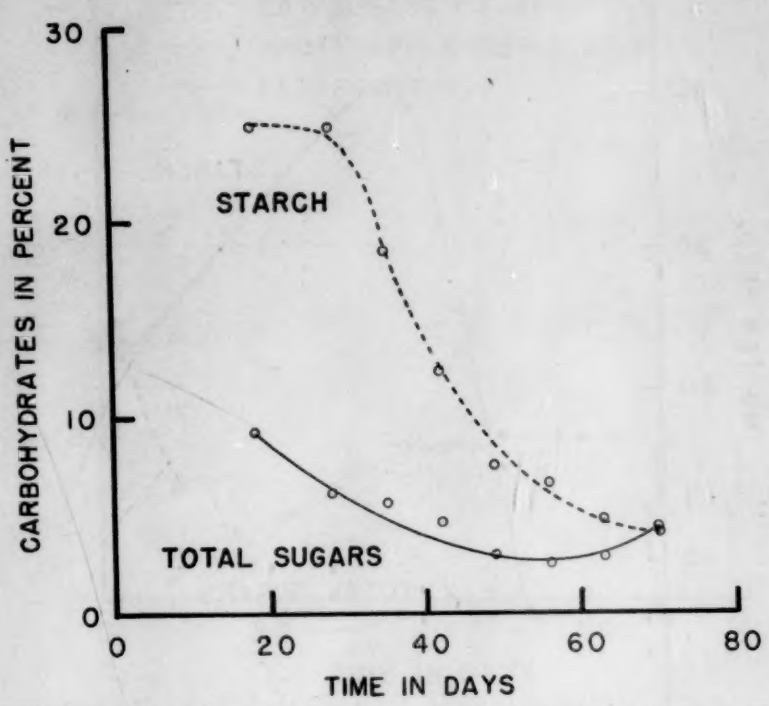

FIG. 6. Carbohydrates in Virginia Bunch seed on a percentage dry weight basis, 1948.

As the seeds developed, both the fat-carbohydrate and protein-carbohydrate ratios increased as maturity was approached. As already reported (3), fat increases at the expense of the carbohydrate fractions; the protein-carbohydrate ratio (fig. 8) indicates the probability of a similar relationship. Although the 70th day sample was somewhat questionable, as previously mentioned, both the fat-carbohydrate and the protein-carbohydrate ratios decreased at over-maturity. This decrease was very abrupt in the fat-carbohydrate ratio due to both a decline in fat and a rise in suerose. The fat-protein ratio increased quite rapidly to mid-development of the seed, slowly increased to the 49th day, and then became practically constant. Evidently

a few days after the ratio of fat-protein attains a value of approximately 2.0, maturity is indicated, as shown by results of both years. The ratios involving carbohydrates fluctuated more widely during the two seasons.

With the exception of moisture, the results obtained from Virginia Bunch shells in 1948 are given on a percentage dry weight basis in table V. The hemicellulose fraction increased as the shel's matured. All other determined constituents decreased with the exception of crude fat which was higher at

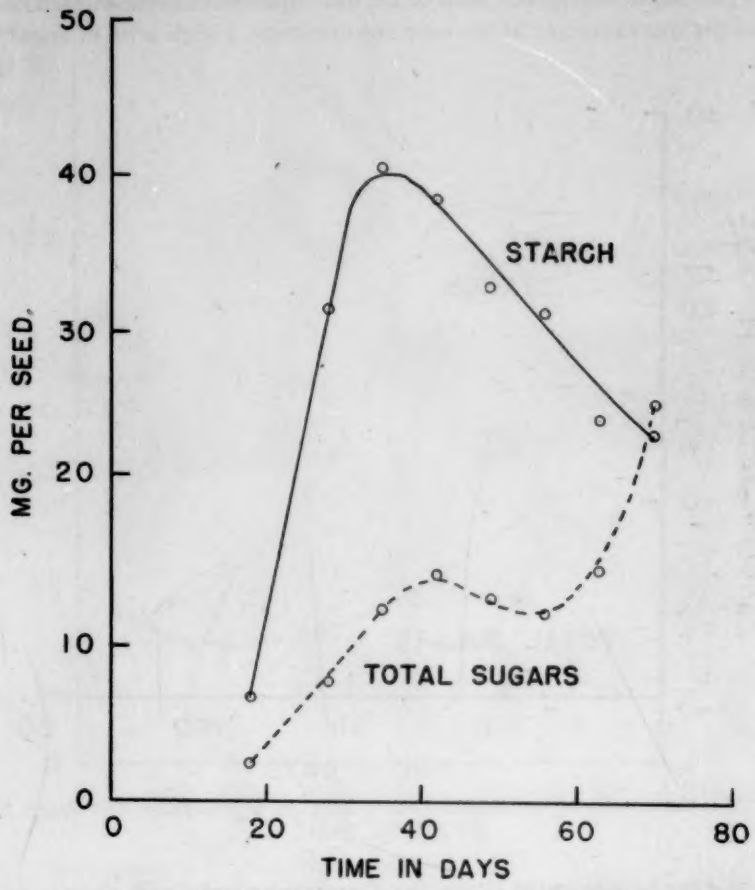

FIG. 7. Carbohydrates in Virginia Bunch seed on a weight per seed basis, 1948.

the 70th day sampling. The percentages of soluble sugars decreased rapidly in the young developing shells and the starch which was relatively low in the very young shells disappeared almost entirely by the 49th day after tagging.

Discussion

Color development in the testa was used as one of the several guides for judging the stage of development of the seeds during the 1947 season and was considered as a possible practical test for estimating maturity. However,

color of the testae was found to vary considerably in amount, shade, and distribution so that this characteristic appears to be of minor value as an indication of maturity. Probably the most reliable and simple method of determining maturity includes: a combination of texture of the seed, color of testa, tightness with which the seed is held by the shell, absence of fleshy material and change of color on the inner side of the shell, and gross appearance of the outside of the shell.

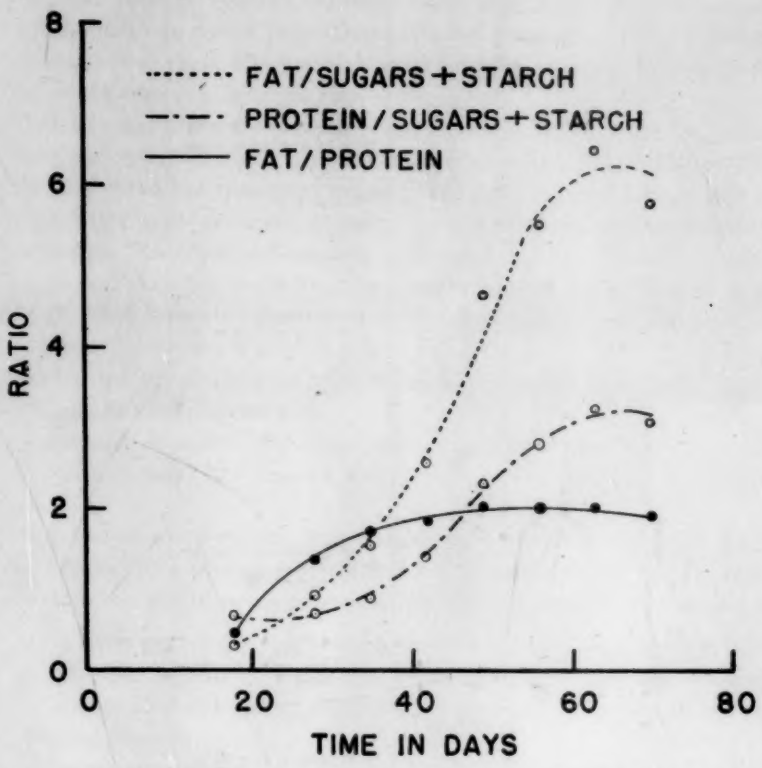

FIG. 8. Ratios of fat, protein, and carbohydrates in Virginia Bunch seed, 1948.

The Virginia Bunch plants were tagged July 19, 1948, when the first and most fruitful growth of the gynophores appeared; 70 days elapsed between planting and the date the tagged gynophores entered the soil. About 10–15 days were required for the gynophores to enter the soil after the flowers appeared, however, considerable variation in time was noted as has been already reported by PATEL and SESHADRI (**7**). Increases in dry weight, fat, and protein in the seed followed curves sigmoid in shape, typical of plant development, the curves tending to flatten as maturity approached. On this evidence as well as by organoleptic tests, 60 $\pm$ 7 days were required for peanuts to mature under the conditions of these experiments. Time required for maturity probably varies considerably with the season. Evidence of over-

TABLE V

COMPOSITION OF VIRGINIA BUNCH SHELLS—1948.

DAYS AFTER TAGGING	MOISTURE FRESH BASIS	CRUDE FAT DRY BASIS	CRUDE PROTEIN DRY BASIS	REDUCING SUGARS DRY BASIS	SUCROSE DRY BASIS	STARCH DRY BASIS	HEMICELLULOSE FRACTION DRY BASIS
No.	%	%	%	%	%	%	%
18	89.6	3.8	17.4	15.9	14.6	1.3	8.0
28	81.9	1.6	14.6	8.6	9.6	1.9	9.0
35	68.8	0.9	16.1	1.7	5.5	1.4	9.9
42	62.6	0.6	12.2	0.5	5.1	0.3	10.4
49	59.3	0.5	10.9	0.3	3.4	trace	9.9
56	57.3	1.1	7.5	0.4	3.6	trace	10.5
63	51.9	0.8	7.9	0.4	3.9	trace	11.0
70	43.4	2.3	5.7	0.3	2.5	trace	10.1

maturity as shown by browning of both the testa and inside of the shell occurred when peanuts were left in the soil 70 days after tagging (140 days after planting).

Summary

The chemical composition of developing Virginia Bunch peanut seed, for two seasons (1947 and 1948) is recorded on a percentage basis as well as a weight per seed basis.

Fat and protein content increase only slightly on a percentage basis during the last two weeks preceding maturity; however, when calculated on a weight per seed basis, these constituents increase considerably since the dry matter of the seed continues to rise.

With the exception of reducing sugars present in the early stages in small amounts and later only in traces, sucrose is the only soluble sugar in the seed and is considered the transport sugar. The percentage of sucrose decreases as the seed develops; however, at maturity a slight increase in content of this sugar occurs. Starch also decreases as the seed ripens. The total percentage of sugars and starch approaches a constant value in the fortnight preceding maturity. The hemicellulose fraction increases throughout the development of the seed.

The ratios of protein-carbohydrate, fat-carbohydrate, and fat-protein increase as the seed develops.

In the shell, hemicellulose increases throughout development; while the percentages of total dry matter, fat, crude protein, sugars, and starch decrease.

From one season's results, it appears that Virginia Bunch peanuts require 130 ± 7 days from planting or 60 ± 7 days from the entrance of the gynophores into the soil to produce seed at the optimum stage for harvesting.

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BASES FOR THE PREDICTION OF CORN YIELDS[1]

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(WITH SIX FIGURES)

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Introduction

Accurate crop yield estimates at the earliest possible date are of major importance to producers, handlers, warehousers, railroads, and consumers, whose plans and operations depend upon the knowledge of present and future stocks of their commodity.

Past attempts at crop prediction have been largely of two types. The Crop Reporting Board of the U. S. Department of Agriculture has prepared its reports from questionnaires sent to large numbers of correspondents. The average of many individual estimates, corrected on the basis of past experience, has been, and probably still is, the most reliable prediction method available. It is obvious, however, that supplementary, objective measurements would greatly increase the value of present estimates. The second method used in the past has attempted to supply objective predictions from Weather Bureau data, and many reports have been published on the correlations between various climatic factors, particularly seasonal rainfall and temperatures, and crop yields (**5**).

Statistical studies of published weather and crop records have not proved to be a promising basis for crop yield predictions. Consequently a Crop-Weather-Yield Project was set up by the Agricultural Marketing Service, cooperating with various State Agricultural Experiment Stations, to determine more specifically the physiological effects of climate on plant development. This project was intended "To improve the regular forecasts by discovering and applying relationships between yield and weather factors, and to include in an objective basis for forecasting the relationship of structural counts, measurements, and phenological records with yields, either separately or with weather factors." The work with corn was started in 1938 and centered in the Iowa Agricultural Experiment Station with the Statistics, Botany and Plant Pathology, Agronomy and other Sections cooperating. Detailed studies of crop development and micro- as well as macroclimate were made on the station plots at Ames in 1938, 1939 and 1940. The results of these studies have been summarized by Bair (**2, 3**). Less detailed studies were made at other corn-belt Stations in 1939 and 1940, a part of the results of which have been published by Keller (**7**).

[1] Journal Paper No. J1669, Iowa Agricultural Experiment Station in cooperation with the Bureau of Agricultural Economics, Division of Crop and Livestock Estimates, U. S. Dept. of Agriculture. Projects 610 and 678.

When it became evident that the Experiment Station plots at Ames formed too narrow a base for the development of correlations and regressions of preharvest measurements and yield, plots were established in the fields of cooperating Story County farmers to obtain moderately intensive records from fields specifically chosen to give a wide variation in soil fertility and yield.

The present report is based upon these studies and is an attempt to determine the preharvest measurements most likely to be of value in yield prediction, together with practical considerations of methods of collecting such data under field conditions. It is not assumed that the equations developed from these data will be of universal application. The principles and methods used, however, are thought to form bases for the prediction of corn yields.

Methods

Twenty-five areas of 8×8 hills were selected in farmers' fields near Ames in 1941 and 20 areas in 1942. These areas were chosen to represent poor, average and good yields and were not necessarily representative of the fields of which they were a part. Each experimental area was divided into 16 plots of four hills each. Half of these plots were used for all those measurements which would not affect their value as a record of final yield. The remainder were used for early harvests, early tests of shucked against unshucked ear measurements, etc.

All of the fields in 1941 and all but one in 1942 were planted with hybrid seed. Eleven varieties were included in 1941 of which Ia-939, planted on eight fields, was the most popular; 1942 varieties were comparable. Soils varied from Dickinson sand, a terrace soil of low yielding capacity, through Wabash, Webster and Clarion sandy loams to silt and clay loams. The plots thus included a cross section of central Iowa varieties and soils.

Measurements were made in four groups: measurements of stand, leaf area, height, basal stalk diameter, and date of 50% silking in July or early August; ear counts and ear growth measurements in August; ear size with and without shucks and early harvest yields in September, and final ear sizes and harvests in October. Actual dates of measurements were based upon stage of development, calculated from date of 50% silking, and varied between fields and between years by as much as two weeks. Stalk and ear diameters were taken with calipers. Stalk diameters were taken as maximum, or maximum and minimum diameters of the somewhat flattened stalks, in the first internode which was not blocked by brace roots. Ear diameters were taken at the center of the ear, either over the shucks (unshucked) or over the grain (shucked). Stalk height was measured after silking to the ligule of the uppermost leaf and recorded as maximum ligule height. Leaf measurements included number of active leaves per stalk and area (length $\times$ max. width) of the leaf arising at the same node as the upper ear (ear leaf). Ear length was measured with a steel tape as length of cob covered with

developing grain. Some difficulty was experienced in estimating grain position when measuring young ears over the shucks. By September 1 estimates of shucked measurements could be made from the ratios of shucked to unshucked measurements of ears on the extra plots or on adjoining rows. At final harvest both unshucked and shucked measurements were obtained on all ears.

Vegetative measurements

The development of the corn plant can be divided into five reasonably distinct stages (**6, 12**), each with its own responses to weather and its own relation to final yield. These stages are: (a) early vegetative growth from planting to flower differentiation; (b) rapid vegetative growth from a plant height of about 50 cm. to silking; (c) pollination and fertilization; (d) grain production from fertilization to maximum dry weight of the grain; and (e) maturation or drying of grain and stalk.

The first developmental stage includes germination, the seedling stage, and early leaf growth during three or four weeks of independent development of the plant. At the end of the period the plants are knee high, the above ground parts are limited to leaf sheaths and blades, the tassel is microscopically visible at or below the ground surface level, culm development is largely embryonic, but the maximum number of leaves, the maximum number of vascular bundles and the maximum number of ovules on the embryonic ear shoots are already determined (**6**). The potentialities of the plant are therefore established during May and early June, and this first period is of great theoretical importance in yield prediction. Actually, however, weather is seldom limiting during this period except as it affects stand; and soil fertility, which is included in the base norms of crop prediction, is the major factor. Also the corn plant so seldom reaches its maximum potentialities under field conditions, and has such extensive powers of recovery from apparently serious early setbacks, that objective measurements during this period can probably add nothing beyond the stand and seasonal development factors already covered by crop reporter correspondents.

The ability of corn to recover from early unfavorable conditions is illustrated in table I. Full stands of six stalks per hill on eroded Clarion soil were thinned to two stalks and fertilized with 450 lbs. per acre of 4–8–6 fertilizer; (a) at germination; (b) at the end of the early vegetative growth, or (c) at silking when vegetative growth was complete. Plants of the second thinning were significantly smaller than those of the first in stalk size, but were not significantly smaller in yield or ear size. Even when the extreme crowding was continued until silking, the later, more favorable conditions resulted in a significant gain in ear size without any change in stalk diameter.

During a period of four to six weeks following the initiation of the staminate and pistillate inflorescences, the leaf area of the corn plant increases

five to 10 times and the weight of the culm by 50 to 100 times (**12**). The average stem elongation during this grand period of growth may be four inches a day. The number of leaves, nodes and bundles was fixed during the first growth phase; at the end of the second the size of these organs has been determined, and maximum stalk height, stalk diameter and leaf area may now be measured.

Farmers' field plot records

The measurements of the Ames cooperative plots, both those taken at the end of rapid vegetative growth and during ear development are summarized in tables II and III. Iowa corn yields in 1942 are the second highest on record, state average 59.9 bu., and the better growing conditions of the second season are reflected in the 17% greater average yields in table III of the fields selected along the same highway route. None of the vegetative measurements of average height, stalk diameter or stand predicted this greater yield. In fact it did not show fully in any measurement before final dry weight of ears. We could assume from this result, remembering the

TABLE I

DEVELOPMENT OF CORN PLANTS CROWDED FOR VARYING PORTIONS OF THE GROWING SEASON

TREATMENT	AVERAGE STALK DIAM., CM.	BARREN STALKS, %	TOTAL YIELD IN BU. ACRE	EAR WT. GM.	EAR WT. GAIN OVER CKS., %
1. Ck—6 stalks—unfertilized	1.60	8.5	112.6	227.5	.....
2. 6 stalks—fertilized	1.66	9.2	117.8	239.0	5
3. Thinned to 2 stalks at silking	1.63	2.2	57.6	295.0	30
4. Thinned at knee high	1.97	1.3	73.2	387.0	70
5. Thinned at germination	2.32	0.0	76.0	390.5	72

recovery of the late thinned plants shown in table I, that the yield differences in 1941 and 1942 were due to conditions acting upon ear development after an average date of August 1. In other words the yield potentialities of the two crops were identical at this stage. For comparison, the August 1 yield predictions for Iowa in the two years were 52.0 bu. in 1941 and 53.0 bu. in 1942. Final, revised yields for the two years were 51.0 and 59.9 bu.

In spite of the apparent discrepancies between vegetative growth and yields of the two years, correlations between measurements on individual plots and yields were high and statistically significant in every comparison (table IV). No leaf measurements were made in 1942 so the table is incomplete in leaf correlations. Maximum ligule (stalk) height gave correlations with yield of $r = 0.79$ and 0.76 in the two years. In 1941 the inclusion of stand in a multiple correlation with height and yield gave a highly significant increase in the correlation coefficient. In 1942, perhaps because some poorly tended fields were thickly planted, stand did not appear to have been a factor in yields, even though average stands for the two seasons were comparable.

TABLE II

MEASUREMENTS ON FARMER'S FIELD PLOTS—1941.

PLOT	VARIETY*	YIELD,† BU./A.	MAX. LIGULE HT., CM.	MAX. STALK DIAM., CM.	PLANTS PER HILL†	EARS PER PLANT†	SEPT. 1†† EAR SIZE, (L × D)	FINAL§ EAR SIZE	FINAL DRY WT. EARS, LBS.
1	Pion.-334	70.3	182	2.16	3.09	0.95	90.4	77.7	.290
2	Iow.-A.Q.	53.4	191	2.39	2.44	0.89	114.4	88.2	.301
3	Dek.-827	79.8	236	3.17	2.59	0.78	132.4	111.3	.477
4	Dek.-827	68.5	207	3.05	1.75	1.03	127.0	102.9	.459
5	Iow.-019	46.1	163	1.89	2.94	0.70	100.9	74.4	.271
6	Dek.-606	82.2	204	2.46	3.03	0.89	107.7	87.4	.371
7	Dek.-606	71.3	197	2.47	2.88	0.94	101.2	81.2	.318
8	Pion.-332	89.2	279	3.25	2.00	1.12	139.6	103.9	.490
9	Dek.-?	80.2	200	2.63	2.91	0.93	120.0	94.8	.358
10	Pion.-307	108.7	230	3.10	2.72	1.06	131.2	100.9	.459
11	U.S.-44	73.4	212	2.50	2.53	0.85	127.4	97.9	.413
12	Ia.-939	77.6	205	2.39	2.78	0.97	115.7	91.0	.351
13	Pion.-307	31.9	139	1.80	2.31	0.84	78.2	59.8	.200
14	Pion.-307	111.8	232	3.43	2.59	1.30	119.0	.....	.362
15	Ia.-939	32.4	150	2.40	2.47	0.89	61.6	53.6	.180
16	Ia.-939	59.4	191	2.33	2.72	0.95	93.4	74.5	.278
17	Ia.-939	43.6	156	1.97	2.25	0.97	100.8	73.3	.242
18	Ia.-942	31.4	173	2.25	1.91	0.90	87.0	65.2	.222
19	Ia.-942	76.0	220	2.70	2.16	0.95	131.6	103.8	.447
20	Ia.-939	115.8	221	2.52	2.94	0.96	131.3	108.5	.495
21	Ind.-750	87.8	204	2.70	2.59	1.02	112.5	96.2	.405
22	Ind.-750	54.6	175	2.53	2.62	0.83	92.5	77.7	.304
23	Ia.-939	47.7	168	2.01	4.09	0.77	73.5	59.3	.184
24	Ia.-939	88.2	194	2.19	4.09	0.75	107.9	88.6	.350
25	Ia.-939	102.5	207	2.23	3.25	0.98	114.4	94.5	.392
Average		71.3	198	2.50	2.71	0.93	108.5	86.2	.345

* Pion. = Pioneer; DeK. = DeKalb; Iow. = Iowealth.
† Adjusted to 40 inch spacing.
†† Over shucks at time of max. ear size—L × D = cm^2.
§ Shucked measurements in field about October 15.

TABLE III

MEASUREMENTS ON FARMER'S FIELD PLOTS—1942.

PLOT	YIELD,† BU./A.	MAX. LIGULE HT., CM.	MAX. STALK DIAM., CM.	PLANTS PER HILL†	EARS PER PLANT	SEPT. 1 EAR SIZE†† (L×D)	FINAL EAR SIZE§	FINAL DRY WT. EARS, LBS.
1	135.5	228	3.04	3.12	0.93	131.1	112.8	.569
2	105.9	217	2.63	2.24	1.10	144.3	118.1	.516
3	44.7	179	1.73	2.24	.98	83.8	70.8	.242
4	71.2	193	2.49	2.12	1.06	121.5	75.9	.384
5	89.2	207	2.60	2.34	1.12	136.9	109.2	.433
6	95.1	203	2.72	2.14	1.03	135.8	113.7	.525
7	74.7	187	2.24	2.47	1.01	104.7	86.1	.363
8	99.2	209	2.61	2.41	1.03	151.9	109.0	.489
9	83.7	207	2.39	2.23	1.02	121.1	99.0	.448
10	103.1	227	2.83	2.26	.97	129.7	112.5	.569
11	102.8	215	2.68	2.20	1.01	140.9	119.0	.559
12	87.9	192	2.27	2.70	.99	120.1	98.3	.399
13	98.6	212	2.84	2.47	1.06	129.0	110.8	.498
14	66.3	171	2.42	2.02	1.04	118.5	91.4	.381
15	50.3	230	2.03	3.15	.84	88.0	68.5	.231
16	44.5	170	1.64	2.94	1.00	77.3	56.0	.184
17	56.6	174	2.25	2.16	1.01	98.0	78.1	.314
18	146.5	241	3.05	3.47	1.05	119.9	101.4	.513
19	93.5	201	2.59	2.14	1.03	133.6	109.0	.502
20	24.1	179	1.85	2.06	.93	72.2	52.2	.167
Av.	83.7	202	2.44	2.44	1.01	117.9	94.6	.414
Per cent. 1941	117.4	102.0	97.6	97.6	108.6	108.7	109.7	120.0

† Adjusted to 40 inch spacing.
†† Over shucks at time of max. ear size—L × D = cm^2.
§ Shucked measurements in field about October 15.

In 1941 maximum stalk diameter gave the poorest correlation with yield ($r = 0.61$) of any vegetative character measured; in 1942 stalk diameter gave the highest correlation ($r = 0.92$). The difference is considered to be confirmation of the generally more favorable late season in 1942 so that the yields were more nearly proportional to the maximum capabilities of the plants as determined by the factors associated with stalk size—bundle number, root number, leaf area, etc. Striking evidence for such a relationship was obtained from the 1941 thinning experiment. The regressions of ear weight on stalk diameter for the thinning treatments of table I are shown in figure 1. The plants thinned at emergence to two plants per hill produced the largest stalks—so large that they were not limiting for yield and so showed no correlation. With later thinning, or no thinning, stalk diameters became limiting for yield and showed significant correlations with ear weight for all plots.

TABLE IV

CORRELATIONS AMONG VEGETATIVE MEASUREMENTS AND BETWEEN VEGETATIVE MEASUREMENTS AT SILKING, AND YIELD

LINEAR RELATION	1941 DATA, CORRELATION* COEFFICIENT	DEGREES FREEDOM	1942 DATA, CORRELATION* COEFFICIENT	DEGREES FREEDOM
1. Max. ligule height and stalk diameter	.828	23	.720	18
2. Max. ligule height and min. stalk diameter	.881	23	.....	.....
3. Max. ligule height and yield	.791	23	.760	18
4. Max. stalk diameter and yield	.614	23	.921	18
5. Number of leaves and yield	.700	23	.....	.....
6. Ear leaf (L×W) and yield	.722	23	.....	.....
7. Max. ligule height, mx. stalk diameter and yield	.794	22	.932	17
8. Max. ligule height, number of leaves and yield	.816	22	.....	.....
9. Max. ligule height, stand and yield	.872	22	.761	17
10. Ear leaf (L×W), number of ears and yield	.810	22	.....	.....

* All correlations shown highly significant, beyond 0.01 level of probability.

The data of table IV and figure 1 suggest at once the possibilities and limitations of vegetative measurements in crop prediction. The limitations are inherent in the fact that none of the marketable grain crop has been produced when vegetative growth is complete. Such growth represents potentialities whose realization is dependent upon subsequent environmental conditions as well as upon the genetic constitution of the plants.

Of the four practicable vegetative measurements, stand, height, stalk diameter, and leaf area, stand should probably receive first attention. Stand counts will, however, be of little value until regression curves for modern varieties and varying climatic sequences have been developed. Attempts to apply specific formulae to stand counts will be complicated by the geometric

factors involved, with yields tending to decrease above optimum stands, and responses varying with season and soil fertility. Recent work by NELSON and DUMENIL (**11**) indicates the responses to be expected. Corn planted at a rate of 2, 3, and 4 stalks per hill gave yields of 59, 67 and 62 bu. per acre respectively with hill fertilizer treatment only, and yields of 71, 83 and 92 bu. per acre with hill fertilizer plus 600 lbs. per acre of 8–8–8 fertilizer. On Webster clay loam with no fertilizer, yields decreased when a stand of five stalks per hill was used instead of four but on fertilized soil yield increased with the heavier stand. This same relationship was found for Clarion loam and Wabash silt loam.

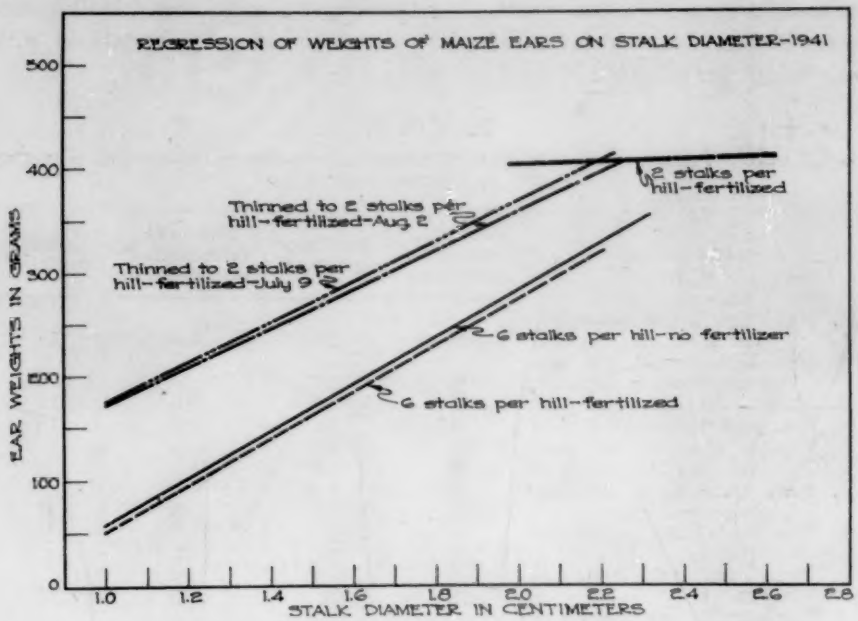

FIG. 1. Regression of ear weight on stalk diameter.

Stalk height and diameter both show varietal differences not closely associated with yield. Within a variety or group of varieties, stalk diameter might be expected on the average to show a higher correlation with yield than height which is markedly influenced by simple internode elongation. Some of our observations suggest, however, that stalk height is a better index of yield within varieties than its physiology would predict. Internode elongation is particularly sensitive to moisture and temperature during the month before tasseling, and is affected by growth hormone production during the development of the inflorescence (**18**). To the extent that July weather is a major factor in corn production, and to the further extent that favorable July weather means favorable soil moisture reserves, stalk height will have a yield prediction value beyond its theoretical physiological role.

Leaf area is a major factor in total dry matter production by the corn plant (**19**) since rates of photosynthesis per unit of leaf area do not vary systemically, even between inbred and hybrid lines. Marked loss of leaves during midseason drought will undoubtedly reduce yields, although the estimation of leaf effects will be complicated by failure of pollination and

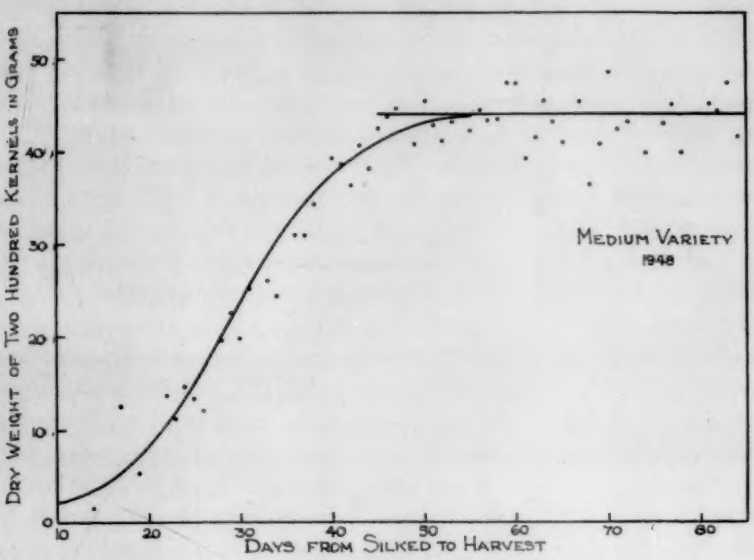

Fig. 2. Accumulation of dry matter in the grain of corn.

embryo development. In the absence of drought injury, leaf area tends to be rather closely correlated with stalk diameter and height, which are more readily measured and would thus be given preference in an action program.

Ear measurements

In contrast to vegetative measurements which indicate the potentialities of the plant, ear measurements begin to measure yield itself. Ear development divides naturally into four phases, the first three of which affect yield directly and the fourth is important in crop prediction in relation to possible frost injury.

Ear shoot development, pollen formation, and fertilization may be thrown together as the first phase; a highly important and vulnerable one in the production of the corn crop. Fertilization is followed by three weeks of rapid growth of ear shoot, shucks, cob and young kernels. The overshuck dimensions of the ear reach their maximum in this period (cf. fig. 3). The ear growth phase is followed by four weeks of translocation and filling of the kernels to maximum size and weight at 30 or 40% moisture (**15**), and finally the physiologically mature crop dries with a minimum of chemical change to harvest maturity at about 20% moisture.

THE FERTILIZATION PERIOD

The development of the young ear shoots and tassels is again primarily growth (**10**). Temperatures above 90° F slow development, particularly when accompanied by moisture deficiencies. Mineral nutrients, especially the protein-building elements nitrogen and phosphorus, should be present in abundance, but their supply, except as it is affected by soil moisture, is included in the base norms of the crop prediction service. With open-pollinated varieties unfavorable conditions just preceding tasseling result in increasing percentages of stalks that fail to develop ear shoots to the silking stage. Hybrid varieties have a much stronger inherent tendency to form ear shoot, but one of the writers (**15**) found silking slowed three to five days and silking time spread out by the dry weather of 1947, until 6% of the stalks of a good hybrid variety failed to silk and another 4% silked too late to be pollinated. In addition, a large percentage of the ears were only partially filled in 1947. In the favorable season of 1948 total barren stalks amounted to only 3%.

Tassels rarely fail to open unless their embryonic development has been prevented by hormone type weed sprays (**14, 17**), but the tassels may blast in hot dry weather or pollen may be so short lived as to result in poor fertilization. BAIR and LOOMIS (**4**) found germinating corn pollen very susceptible to either too much or too little moisture. In unpublished data these workers found midday pollination with immediate bagging satisfactory at temperatures up to 100° F. Midday pollination with delayed bagging, however, gave only 50% fertilization because of drying of the pollen on the silks. This difficulty is avoided in the field by the tendency for pollen to be shed early in the morning. When silk temperatures were raised to 120° F immediately after pollination, fertilization failed, as it did with all lots of pollen heated to 125° F before pollination. These temperatures are above normal for corn belt fields but are possible silk temperatures with direct sunshine and air temperatures on the order of 110° F. It seems probable, however, that drying is more closely correlated with failure of pollination than heating. Spraying the silks with water 30 minutes after pollination resulted in burst pollen grains and poor or no fertilization.

In addition to failures in fertilization, young ovules, especially near the tip of the ear, may abort within a week or two after fertilization. Abortion is considered to be a nutritional response, but the relative importance of soil nutrient deficiencies, carbohydrate supplies, moisture stress, and perhaps the direct effects of high temperatures, has not been determined. Additional yield losses may result from incomplete development of tip kernels, or from failure of all the kernels to develop to maximum size.

The production of barren stalks by unfavorable weather conditions can be determined within two weeks or less after the silking period, but the incomplete pollinations and abortions which result in small and malformed ears are harder to estimate, and typically are determinable only at the end of the second or even the third growth phase.

Ear growth

Fertilization initiates rapid cell division and enlargement in the kernels, and is necessary for normal differentiation of the cob (**16**). Bagged cobs on which grain production is prevented may be normal in size, but are light in weight and weak in structure with low percentages of cellulose and lignin. Within two or three days after fertilization embryo growth begins to dominate the entire development of the plant (**8, 9**). Before this time the ear shoot is weak in its ability to compete for foods; afterwards foods move toward the ear shoot from all parts of the plant and the forces acting may be so strong as to result in starvation and early death of the roots, and in weakening, even of the leaves.

Cell division and enlargement are the dominant phases of ear development for approximately three weeks. Unpublished work by Dr. J. E. Sass of the Department of Botany at Ames, shows that cell division in the endosperm is completed in about two weeks, in the embryo, except the plumule, in three weeks, while in the plumule some division continues past the fourth week. The cells of the grain enlarge as they divide, but have reached less than half of their maximum size at the end of this period. Because of the rapid growth and high respiration of the tissues, dry matter percentages increase slowly and reach about 30%, with ears in the late milk stage, at the end of the second period. The cob has now reached approximately its final size, but is not yet fully differentiated and shows higher percentages of sugars and lower proportions of cellulose and lignin than it will later (**16**).

Translocation and filling of the ears

The third stage is dominated by translocation, with some cell enlargement but little or no cell division, until maximum ear size and weight are reached, the first typically six and the second seven or seven and one-half weeks after pollination.

The gain in dry weight of the developing corn kernels of Pioneer 340, a medium maturing variety, is shown in figure 2. Few data points are included for the growth phase since these kernels were too watery to handle well. It is clear, however, that the total dry weight of the 3-week kernels was only 10 or 15% of the maximum weight reached four weeks later. This gain is accompanied by a doubling or more of the size of the kernels and an increase in percentage dry matter, on the wet weight basis, from about 30 to 60 or 70. The cob hardens and decreases in water content during the same time, but shows no significant change in dry weight.

This filling period is less sensitive to weather factors than either of the preceding periods, since it involves less cell division and a lower level of physiological activity. Photosynthesis is not likely to be reduced below normal unless the plants wilt during the day (**19**), or the lower leaves fire because of drought. Translocation and storage also are not particularly sensi-

tive except to considerable moisture stresses. On the other hand, some 85% of the marketable crop is produced during these four weeks, and even small changes in rates can affect final yields. We have already suggested that the 17% gain in final yields of 1942 over 1941, shown in tables II and III, was due to the more favorable conditions for filling in 1942. In 1947 the weather was dry after midseason and kernel size was reduced and translocation slowed by moisture stresses and premature drying of the ears (**13**).

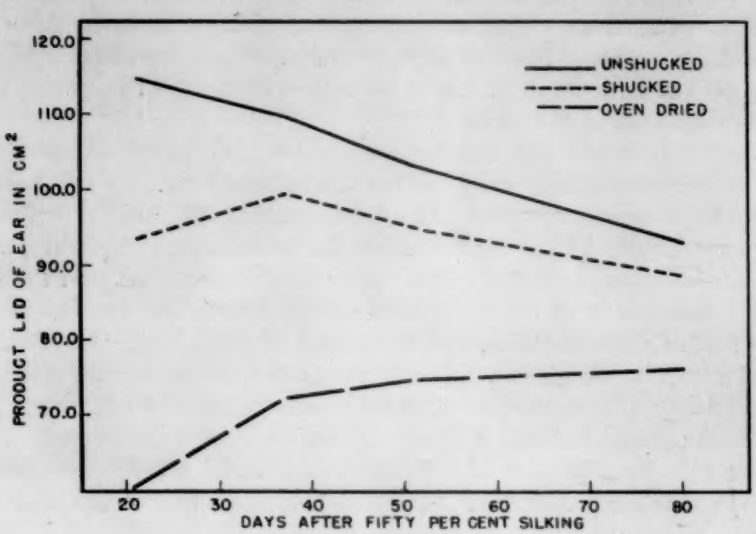

FIG. 3. Changes in the size of maturing ears of corn.

SHAW (**15**) has found a remarkable constancy in the length of the ear-growth and filling periods, with no significant differences between early and late varieties grown at Ames in 1947 and 1948. Early varieties reached 75% silking two weeks before the late, but both reached maximum dry weight of kernels 50 or 52 days after silking. The moisture content of the early varieties at this physiological maturity was 28 to 30, while that of the late was 40%. From the grower's standpoint the early corn was nearer cribbing maturity at the end of its filling period, but it was not ahead in percentage of crop produced at any given time after silking.

Ear size as a possible index of yield is a complex variable. The data of figure 3 show that over-shuck measurements reached their maximum at three weeks after silking or before and then declined steadily as the shucks dried. Under-shuck measurements reached maximum at five weeks and declined as the cobs dried and the kernels dented. Oven dry ears showed considerable shrinkage at all times, but reached approximately maximum size at the 7-week date which was established as the point of maximum kernel dry weight.

Field drying

From approximately seven weeks after silking the maturation of the corn crop is a simple drying process subject to weather conditions, and slowed somewhat by the shuck covering of the ear. Freezing during this period will not affect total yields but may reduce viability and keeping qualities (**13**). Frozen corn dries more slowly under the same conditions than unfrozen (**1**), and in addition the weather in soft-corn seasons is usually unfavorable for drying. Corn at 30% or even at 40% moisture, however, is not injured by periods of two or three hours at temperatures above 20° F (**13**), so that frosts, as contrasted to freezes, do not injure the physiologically mature crop.

Estimation of yields from ear measurements

The preceding discussion shows that fairly definite ear counts and measurements are available three weeks after silking, or about August 20 at Ames in an average year. Ear measurement data could then be used for the September 1 and later predictions. The earliest measurements would be over-shuck and subject to fairly large errors; by September 15, however, accurate estimates of final ear number and weight are possible.

Correlations

The correlations of various ear measurements with yields are shown in table V. Even on August 15, shortly after the period of rapid ear growth for the earlier fields and during this period for the later, over-shuck measurements within fields showed high correlations with final weights per ear for the same fields and, when corrected for ear number, with per acre yields. In general, correlations increased in value at the later dates. Surprisingly the correlations for shucked (under-shuck) ear measurements were not significantly higher than for measurements taken over the shucks. The regressions for yield on over-shuck measurements are more difficult to correct for moisture percentages because of their greater change in slope than those for shucked ears, but either measurement would seem to be usable. As shown by the September 15 and final harvest the maximum correlations to be expected are slightly under .98. This is the correlation between ear size and dry weight per ear of the same ears.

A September 15 harvest was used to obtain a comparison between early pre-harvest sampling and final yield. The average estimated yield for September 15 was 66 bushels, while the final harvest was 68 bushels per acre. Deviations between the two harvests on individual fields ranged from – 12.2 to + 18.1 bushels, with the extreme deviations due to a difference in the number of ears obtained from small plots. The average dry weight per ear was .348 pounds on September 15 and .349 pounds at final harvest. Correlations for this early harvest are shown in table VI.

TABLE V

CORRELATIONS ON EAR MEASUREMENTS. 1941 AND 1942.

FACTORS	DEGREES OF FREEDOM	CORRELATION COEFFICIENT
1941		
August 15 (63.7% moisture)		
Unshucked (L×D) and final yield	16	.526
Unshucked (L×D), number of ears and final yield	15	.903
Unshucked (L×D) and final dry weight per ear	23	.936
September 1 (51.6% moisture)		
Unshucked (L×D) and final yield	23	.746
Unshucked (L×D), number of ears and final yield	22	.958
Unshucked (L×D) and final dry weight per ear	23	.942
Shucked (L×D) and final dry weight per ear	23	.943
[Shucked (L×D) × number of ears] and final yield	23	.962
[Unshucked (L×D) × number of ears] and final yield	23	.967
September 15 (38.5% moisture)		
Unshucked (L×D) and final yield	23	.786
Unshucked (L×D), number of ears and final yield	22	.958
Unshucked (L×D) and final dry weight per ear	23	.970
Shucked (L×D) and final dry weight per ear	23	.969
[Shucked (L×D) × number of ears] and final yield	23	.972
Shucked (L×D), [shucked (L×D) × moisture per cent] and final dry weight per ear	22	.971
Final harvest (18.4% moisture)		
Unshucked (L×D) and final dry weight per ear	22	.974
Shucked (L×D) and final dry weight per ear	22	.977
[Shucked (L×D) × number of ears] and final yield	22	.967
1942		
September 1 (58.8% moisture)		
Unshucked (L×D) and final yield	18	.784
Unshucked (L×D), number of ears and final yield	17	.917
Unshucked (L×D) and final dry weight per ear	18	.917
Shucked (L×D) and final dry weight per ear	18	.920
[Shucked (L×D) × number of ears] and final yield	18	.906
[Unshucked (L×D) × number of ears] and final yield	18	.929
Final harvest (20.9% moisture)		
Unshucked (L×D) and final dry weight per ear	18	.917
Unshucked (L×D), number of ears and final yield	17	.917
Shucked (L×D) and final dry weight per ear	18	.920
[Shucked (L×D) × number of ears adjusted to unit area basis] and final yield	18	.970

TABLE VI

CORRELATIONS ON EAR MEASUREMENTS. 1941.

FACTORS	D.F.	CORRELATION COEFFICIENT
September 15 harvest		
Unshucked L. and yield	20	.899
Unshucked D. and yield	20	.903
Shucked L. and yield	20	.916
Shucked D. and yield	20	.905
Shucked (L×D) and yield (unadjusted for moisture)	20	.971
Shucked (L×D) and yield (adjusted for moisture)	20	.975
Unshucked L., unshucked D. and yield	19	.971
Shucked L., shucked D. and yield	19	.973

Regressions

Plant measurements are used to obtain regression equations, which may then be used to estimate yields. The regression of yield on shucked ear size (length × diameter) for September 15 collections in 1941 is shown in figure 4.

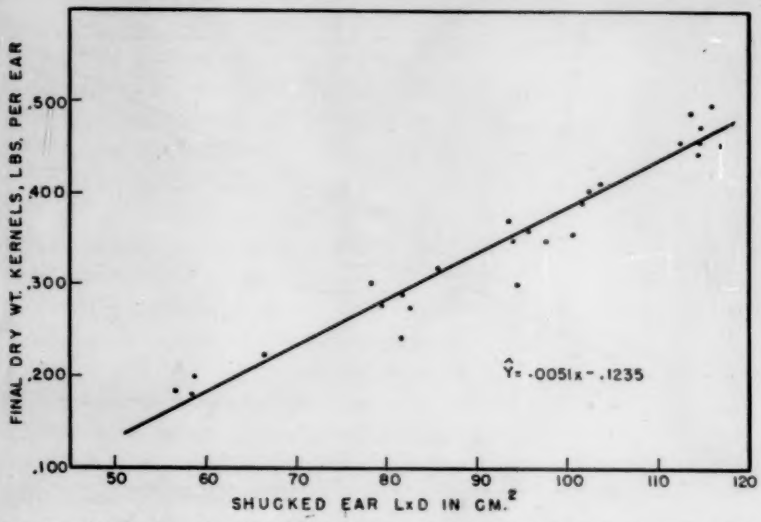

FIG. 4. Regression of weight of grain on ear size; September 15 harvest, 1941.

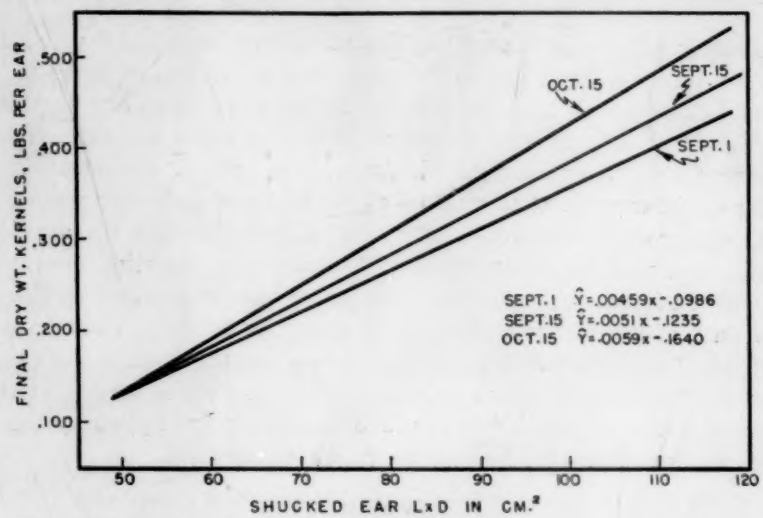

FIG. 5. Regression of weight of grain on ear size at three dates in 1941.

Each data point is the average of 80 ears from a separate field. Linear regression lines for the September 1, September 15 and final harvest dates are shown in figure 5. Shrinkage of the ears gives steeper lines for the later sampling dates, and the proper regression line can be selected only when the

average moisture percentage of the grain is known. For the data of figure 5 moisture percentages were 51.6, 38.5 and 18.4 for the three dates September 1, 15, and October 15. When the dimensions of unshucked ears are used in regression equations, possible differential rates of drying and shrinkage of shucks, grain and cob complicate this moisture correction.

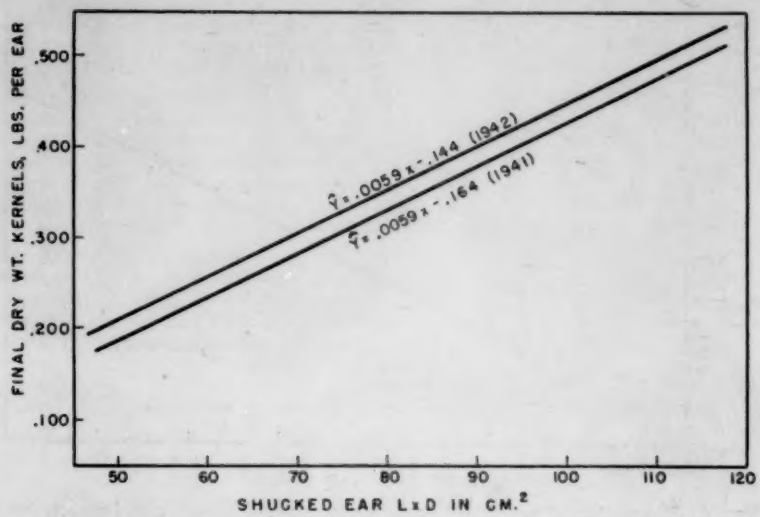

FIG. 6. Regression of weight of grain on ear size at final harvest; 1941 and 1942.

The regression lines for ear size at final harvest (shucked length × diameter) on dry weight per ear in the two years are shown in figure 6. The slopes of the two lines are identical, but the adjusted mean dry weights per ear differed at the 5% level as shown by a covariance analysis. That is, volume or density, or both, of the grain from ears of the same size was greater in the more favorable season of 1942. Since these two years represented rather extreme climatic effects, it is probable that the differences found here would not be exceeded with comparable varieties, even with a wide range of growing conditions, and that a regression based on the combined data of the two years would be generally useful. This regression would be: $\hat{Y} = .0060\,x - .5398$. With this equation the average size ($L \times D$) of the shucked ears, corrected to 20% moisture, would be used to calculate weight of grain in pounds per ear, and this figure multiplied by the estimated number of ears per unit area would give the yield.

Discussion

Yield prediction in the grains is complicated by the growth habits of plants which complete their vegetative development before flowering. As a result, the potentialities of the plants are determined during the first part of the growing season while the marketable crop is produced during the second. This situation is further complicated in corn by the long growing

season required, and by the vulnerability of the crop during its midsummer flowering period. The final yield of the corn crop is thus determined during the five, partially independent periods of: (a) germination and early seedling growth, which determine stand; (b) vegetative growth to tasseling and silking, which determines the absorptive and photosynthetic capacities of the plants; (c) pollination and fertilization, which fix the possible number of kernels per plant; (d) grain growth during a 3-week period of active cell division, which largely fixes the final number of kernels; and (e) filling of the grain during four weeks of translocation and storage, when the actual crop is produced.

Stand is considered in the present questionnaire method of crop prediction. It could be measured more accurately with actual counts by trained personnel in large numbers of fields. The value of such counts would be questionable, however, first because weather conditions after this period have too much affect on yield to enable more than an estimate of yield by any method, and second because the regressions of yield on stand depend upon soil fertility and upon moisture supply. Soil fertility can be predicted, but moisture supply cannot. In dry years or on less fertile soils in the corn belt, maximum yields are obtained with stands of 7,000–10,000 plants per acre, or between two and three stalks per hill. Under the most favorable conditions, yield rises slowly to stands of 20,000 stalks or more, particularly when the plants are drilled in narrow rows. Such heavy stands under unfavorable conditions of moisture and fertility, however, may reduce yields drastically.

Vegetative growth fixes the absorptive, translocation and photosynthetic capacities of the plants and thus their potential yield. Since none of this yield has been produced, however, detailed measurements on a large scale are still unjustified. The correlations shown in table IV indicate the difficulties, as do the regressions of figure 1. Maximum variation in correlation coefficients, $r = 0.6$ to $r = 0.9$, was obtained for the same measurement, stalk diameter and yield, in two seasons. In figure 1 the ear size for stalks of 1.5 cm. diameter varied with stand from about 175 to 275 g. It seems probable that no vegetative measurement would be uniformly valuable enough to justify its collection on a large scale.

Fertilization can be predicted to a limited extent by the general moisture and temperature levels just preceding silking, and can be measured quantitatively within two weeks afterward. Under certain conditions an objective check of correspondents' estimates would be useful and justifiable. Normally this check might be taken three weeks after silking when maximum over-shuck ear size has been attained (fig. 3), and the cell division phases of the crop completed. Only 15% of the weight of the corn crop has been accumulated in the ears at this time (fig. 2), but the critical growth phases are over and an objective measurement, weighted again by current moisture supplies, would have definite prediction value.

Four weeks after the end of the cell division phase of ear growth, and

usually in the last half of September, the crop is made, even though moisture in the grain may run as high as 40%. Accurate yield predictions are now possible and might frequently be valuable, in local areas at least. A procedure used in another phase of the study reported here consists of random sampling of farmer's fields at five to 10 mile intervals. The sample route is planned so that mileage in each area is proportional to acreage. Measurements can consist of as little as a count of the ears on four to six hills and measurements ($L \times D$) of the ears on one hill. An occasional ear is brought to the laboratory in a moisture proof bag for moisture determination and the establishment of the proper regression equation (fig. 5). Calculated yields usually have to be adjusted downward to agree with Assessor's records, probably because of a tendency of growers to underestimate shelling percentages at the time of cribbing.

Summary

Twenty-five areas of 8×8 hills of corn were selected in farmers' fields near Ames, Iowa in 1941, and twenty areas in 1942. Within these areas eight plots of four hills each were used for individual stalk and ear records which started at silking and continued until final harvest in October. The other eight plots were used for moisture samples, comparison of over-shuck and under-shuck ear size, early harvests, etc.

Measurements on each of the record stalks started in late July with stalk diameter, height, area ($L \times W$) of the leaf at the ear node, and continued with records ($L \times D$) of the growth of the ears during August and September, to final ear measurements over- and under-shuck, weights and yields of dry grain for each ear.

Soils varied from Dickinson sand through Clarion, Webster and Wabash sandy loams to silt and clay loams. Yields varied from 24 to 146 bushels an acre.

Correlations between stalk (ligule) height and yield were $r = 0.79$ in 1941 and $r = 0.76$ in 1942. The inclusion of stand in a multiple correlation of height and yield gave a significant increase in the correlation coefficient in 1941, but had no effect in 1942. Stalk diameter gave the poorest correlation with yield of any vegetative measurement in 1941 and the best correlation in 1942. The difference is explained on the basis of the favorable late season weather in 1942 which enabled the plants to reach more nearly their maximum potential development.

Over-shuck ear measurements reached a maximum two or three weeks after silking and then declined as the shucks dried. Under-shuck measurements reached a maximum five weeks after silking, and dry weight of grains, seven to seven and one-half weeks after. The correlation between ear size three weeks after silking and ear size at harvest was $r = 0.94$. Correlations of 0.90 to 0.98 were obtained between all early ear measurements and final weight of ears, or, when corrected for number of ears, between all early ear measurements and yield.

The regressions of yield on ear size ($L \times D$) were as follows in 1941:

September 1 (51.6% moisture) $\hat{Y} = .0046\,x - 0.0986$
September 15 (38.5% moisture) $\hat{Y} = .0051\,x - 0.1235$
October 15 (18.4% moisture) $\hat{Y} = .0059\,x - 0.1640$

As an average of the two years the regression at harvest was: $Y = .0060\,x - 0.5398$.

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HYGROSCOPIC EQUILIBRIUM AND VIABILITY OF NATURALLY AND ARTIFICIALLY DRIED BLUE LUPINE SEEDS

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(WITH EIGHT FIGURES)

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The blue lupine (*Lupinus angustifolius* L.) is grown widely in the southeastern states as a winter cover and green manure crop because it yields more green matter and more harvestable seed per acre than do other soil-protecting and soil-building plants used in this region. Several problems in the production of viable blue lupine seed stock, however, have prevented the broad acceptance which this plant merits. Among these are: (1) the mechanical injury to some seeds inevitably resulting from the practice of threshing the crop from immature pods to avoid dehiscent seed losses in the field; (2) the practical difficulty of providing cool, dry storage facilities during the usually hot, humid summer storage period; and (3) the marked decline in germinability which often occurs even under apparently favorable storage conditions (**7**). Artificial drying and recleaning of seeds at time of harvest have reduced seed losses materially, but the shortage of seed-drying facilities and the necessity for empirical drying methods have delayed the full use of artificial curing potentialities.

Blue lupine seeds mechanically dried to about 10% moisture can be safely stored at somewhat higher temperatures and humidities than can most naturally dried lupine seeds. This may be due to drying effects on seed coat permeability or on hygroscopicity of seed colloids. It is more probable, however, that most field-cured seeds are not dried to a safe storage moisture content (12% or less) and may contain even as much as 18% moisture (**13**) when apparently dry enough to store.

Little work on the physiology of the blue lupine seed has been reported. It often has been noted that the seed is unusually hydrophilic. Blue lupine seeds of 10% moisture when immersed in water may increase over 175% in volume and 165% in moisture content (dry weight basis). Corresponding figures are considerably lower for even such highly hygroscopic seeds as garden peas or beans, where similar treatment produces volume increases of about 125 to 130% and increases in moisture content of about 110%.

McKee and Musil (**13**) found daily moisture fluctuations of as much as 2% in blue lupine seeds exposed to normal atmospheric humidity changes. Fluctuating humidity has been shown by Barton (**2**) to be more harmful than constant humidity of comparable value in its effects on viabilities of certain seeds. McKee and Musil (**13**) also showed that blue lupine seeds take up more than twice as much water from a nearly saturated atmosphere at 35° C as at 20° C. These results are contrasted with those of Karon

and HILLERY (11) who report that the moisture in peanuts was little affected by increasing the temperature from 25° C to 35° C. They confirm numerous observations by growers and seedsmen that blue lupine seeds fluctuate greatly in moisture content during storage, which helps to explain the frequent decline in viability with subsequent heavy losses to some seed growers.

Moisture changes in blue lupine seeds resulting from variations in atmospheric humidity and temperature are characteristic of most seeds, which tend to assume a moisture content in equilibrium with the moisture held by the air at a given temperature and pressure. However, this seed moisture percentage, called the hygroscopic equilibrium, differs widely among seeds of different species of plants both in amount and in rate at which it is reached. It also varies with seeds of the same species or variety, depending upon such factors as age of seeds and conditions of growth, harvest, and storage. There is little basis for predicting the hygroscopic equilibrium of a particular type of seeds, except that oil-rich seeds generally have lower equilibria than those of cereal grains. LARMOUR *et al.* (12) and KARON (9) have shown, however, that if recalculated on a lipid-free basis, the hygroscopic equilibria of several types of seeds are brought into rather close agreement.

The extreme variations among comparable lots of blue lupine seeds in loss of viability when stored under similar conditions, and the unexplained decline in germinability of some seed lots when stored under apparently favorable conditions emphasize the need for fundamental research on response of lupine seeds to their environment. These anomalous results have been noted even under experimental conditions (13). Because of the importance of the hygroscopic equilibrium and its rate of attainment in determining the storage behavior of seed, a series of experiments was begun at the Alabama Agricultural Experiment Station to investigate the relation of the hygroscopic equilibria of both naturally and artificially dried blue lupine seeds to their viability. Three methods of determining the equilibria at different humidities were used: (1) saturated solutions of various salts, (2) sulphuric acid solutions, and (3) an electric hygrometer.

Materials and procedure

SEED SOURCES

NATURALLY DRIED SEEDS.—All experiments in 1947 on hygroscopic equilibria and germinability were run with naturally dried seeds. Samples of field-cured Alabama seeds used in these tests were obtained from Mr. C. P. Storrs of Elmore County, Mr. V. E. Hoover of Baldwin County, and the Wiregrass Substation of the Alabama Agricultural Experiment Station in Henry County. Initial moisture content of both the Storrs and the Hoover seed was 9.0%. The original germinabilities were 95% for the Storrs seed and 46% for the Hoover seed. The Wiregrass Substation sample was from a seed lot originally 85% germinable, but which had been held over concentrated sulphuric acid for eight weeks. It had a moisture content of

3.6% and a viability of 38% (plus 9% "hard" seeds incapable of imbibing water) when used for these experiments. Hygroscopic equilibria were determined only on the Storrs seeds, but germinability tests after storage at the same humidities were also made on the Hoover and Wiregrass Substation seeds.

Four series of moisture equilibria experiments were run on naturally dried seeds in 1948. Two of these tests, in which 6- and 8-weeks storage periods were compared, utilized a commercial grade of seeds obtained from E. L. Story and Sons, Macon County, Alabama. This sample had an initial moisture content of 12.1% and an initial germinability of 90%. The third 1948 experiment, which made use of the electric hygrometer to determine equilibrium moistures, was run with field-cured seeds of various moisture contents harvested from the same field of E. L. Story and Sons, after different drying periods. It was necessary, because of the nature of the electric hygrometric method, to dry some of these seeds to lower moisture contents than those held in equilibrium with normal air. This drying was done at low temperatures (115–140° F) in a forced-draft oven.

In the fourth of the 1948 experiments with naturally cured seeds, where commercial storage conditions were simulated, seeds of four moisture content levels were used to determine their comparative storage properties when held for six months at five relative humidity levels. The range of seed moisture contents chosen was based on information secured from seedsmen, and included both the normal and extreme moisture storage percentages. The four moisture contents thus selected to represent excessive, high, average, and low values were, respectively, 21, 16, 13.5 and 10%. Actual moisture percentages as determined by the Steinlite electric moisture tester for individual samples of the four groups were $20.8 \pm 0.3\%$, $15.9 \pm 0.2\%$, $13.6 \pm 0.1\%$, and $10.05 \pm 0.05\%$. Seeds of all moisture levels had been collected from the same field of E. L. Story and Sons after different drying periods.

ARTIFICIALLY DRIED SEEDS.—Market grade samples of lupine seeds from two commercial seed drying plants were used in an experiment designed to determine possible differences in the total and rate of water absorption between these artificially dried seeds and naturally cured seeds. At the time of first exposure to the various humidities, one drying plant sample had a moisture content of 10.8% and germinability of 97%, and the other a moisture content of 8.9% and a viability of 93%. Unfortunately, it was not possible to run simultaneous tests with naturally cured seeds of the same original sources.

EXPERIMENTAL STORAGE CONDITIONS

HUMIDITY CONTROL.—Solutions of both sulphuric acid and various salts were used to produce the desired relative humidities of the storage air in these experiments. The superiority of saturated solutions of pure salts and of salt mixtures to sulphuric acid solutions in maintaining constant

relative humidities in the closed containers used for hygroscopic equilibrium seed studies was recognized, but the variety of salts necessary to produce the appropriate range of relative humidities was not available for the 1947 studies, or in sufficient quantities for the 1948 test simulating commercial storage. Sulphuric acid solutions were therefore used in these experiments.

In all experiments where the lowest obtainable humidities were desired, concentrated sulphuric acid of 1.834 sp. gr. (18° C) was used to produce initial humidities of very nearly zero. Other relative humidities produced by means of sulphuric acid solutions rather than by salt solutions were obtained by use of the proper hydrometrically determined dilutions (**17**) of the acid with water. In all laboratory storage chambers employing full-strength sulphuric acid or acid mixtures with water, a ratio of weight of liquid to weight of seeds of about 5 : 1 was provided to minimize the changes in relative humidity as the seeds and air came to equilibrium. These changes were evaluated in the 6-months commercial-type storage test by hydrometric measurements of specific gravities of the humidifying solutions both before and after the storage period.

In those tests utilizing saturated solutions of various salts to produce the selected relative humidities, the salts were those used in the extensive hygroscopic equilibria experiments of Karon *et al.* (**10, 11**). Their corresponding relative humidities at 25° C at saturation (**8**) were: LiCl, 11.1%; $KC_2H_3O_2$, 22.5%; $MgCl_2 \cdot 6H_2O$, 32.5%; K_2CO_3, 43.7%; $Na_2Cr_2O_7 \cdot 2H_2O$, 53.3%; $NaNO_2$, 64.4%; NaCl, 75.4%; K_2CrO_4, 86.4%; and $NH_4H_2PO_4$, 92.5%. In both the sulphuric acid and saturated salt solution series, distilled water was used in all cases where a relative humidity of 100% was to be maintained.

The relative humidities selected for experimentation spanned the range of 0 to 100% in those cases where the entire hygroscopic equilibrium curve was to be determined. In those studies where the storage behavior of naturally or artificially dried lupine seeds was to be investigated for only the humidities commonly encountered in storage, the relative humidities were chosen to represent the average range and the extremes (50 to 100%) that normally would occur under summer weather conditions in the southeastern states.

All of the laboratory hygroscopic equilibrium tests were run in sealed glass containers. In most cases, the seeds were suspended over the humidity control liquid in shallow screenwire baskets to permit greatest possible exposure of the seeds to the moisture of the air. In the 8-weeks storage test using sulphuric acid solutions as the humidifying agent, the seeds were similarly suspended in wide-mouth shallow glass vials. The humidity control chambers were not opened throughout the period of storage, except for those in the studies of moisture equilibria of artificially dried seeds. With these latter tests, it was desired to follow the moisture uptake at regular intervals, and the containers were opened weekly to permit weighings of the seeds.

Moisture percentages of these seeds at the 1-, 2-, 3-, and 4-week intervals were calculated from determinations of relative increases in weight of the seeds, based on the assumption that the experimental seeds had the same initial moisture content as seed of the same original source on which moisture percentages had been determined by oven-drying. The equipment used to set up the experiment imitating commercial storage is illustrated

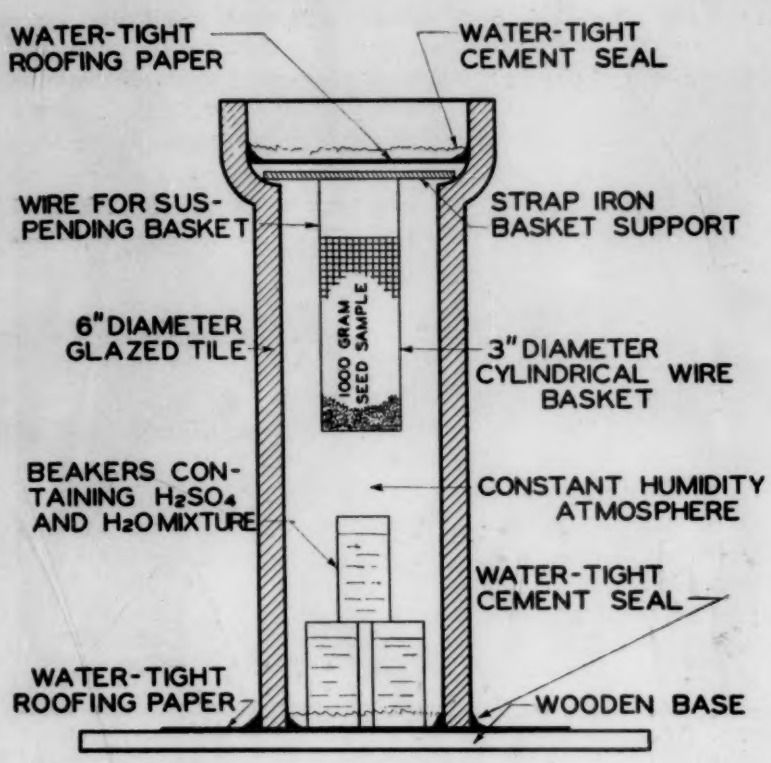

FIG. 1. (A) Chamber and humidifying equipment used for storing blue lupine seeds for six months at various relative humidities.

in figure 1A, where details of seed-sample size and treatment are shown.

STORAGE TEMPERATURES.—Laboratory seed storage tests were run at room temperatures held as nearly as possible at 25° C. In two series of tests using saturated salt solutions for humidity control, and in the determinations of equilibria employing the electric hygrometer, temperatures were carefully held at 25° C by placing the seed storage containers in an insulated cabinet in a constant temperature room. The commercial-type storage test was run in a ventilated shed exposed to daily and seasonal temperature fluctuations.

THE ELECTRIC HYGROMETER.—Determination of the hygroscopic equilibrium by means of the electric hygrometer approaches the problem of mois-

ture exchange from the viewpoint of the equilibrium relative humidities produced in confined air by seeds of various moisture contents rather than of the moisture contents attained by seeds held at various relative humidities. The humidity chamber used for this determination is illustrated in figure 1B, which also shows placement of the seed samples. Each of the 25 equilibrium points determined by this method was made by holding the seeds in the humidity chamber until equilibrium moistures were reached between seeds and air. Equilibria were reached in 24 hours in all cases except for

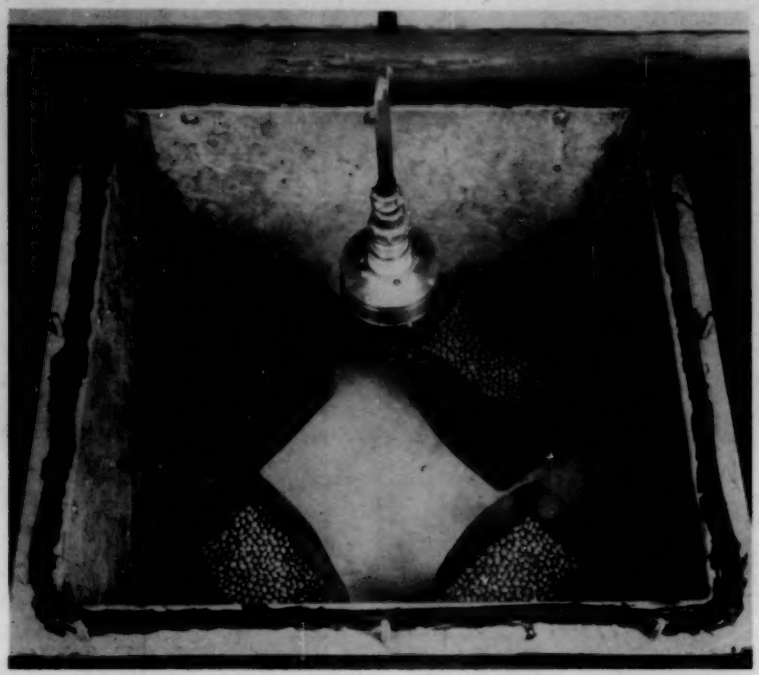

FIG. 1 (B) Moisture-tight box used for determining equilibrium relative humidities produced in confined air by blue lupine seeds of different moisture contents. Sensing element of electric hygrometer is shown in center of lid.

the two lowest humidities, where 72 hours were required. Humidity measurements were made electrically with the hygrometer, a recent development of the Bureau of Standards (5) manufactured by the American Instrument Company.

VIABILITY AND MOISTURE TESTS ON SEEDS

GERMINABILITY TESTS.—All tests of viability of the blue lupine seeds of various experiments were made by placing sextuplicate (in some cases quadruplicate) 25-seed samples with 10 ml. of water on filter paper in previously sterilized Petri plates which were then held at room temperature for 10 days before final counts of germinated seeds were made.

SEED MOISTURE TESTS.—Almost all determinations of moisture percentages held by the lupine seeds both before and after storage treatments were

made by drying representative samples of the seeds to constant weight at 105° C in a forced-draft oven. In the commercial-type storage experiment, where preliminary rapid estimates of seed moisture of a large number of samples were desirable, the Steinlite electric moisture tester was employed. Seed moisture determinations with this instrument agree well with oven measurements except with seeds of high water content.

Results

The hygroscopic equilibria of blue lupine seeds of various initial moisture contents and types of prior handling are presented in tables I–IV, inclusive, and in figures 2 (A, C, D), 3 and 8. Moisture absorption data for other seeds in which equilibria were not reached under all conditions are given in

TABLE I

MOISTURE PERCENTAGES AND GERMINABILITIES OF NATURALLY CURED AND OF CHEMICALLY DRIED BLUE LUPINE SEEDS AFTER STORAGE FOR EIGHT WEEKS AT VARIOUS RELATIVE HUMIDITIES. METHOD OF SULPHURIC ACID SOLUTIONS

RELATIVE HUMIDITY (INITIAL)	FINAL MOISTURE CONTENT* (WET BASIS)	FINAL GERMINABILITY		
		NATURALLY CURED SEEDS		CHEMICALLY DRIED SEEDS†
		INITIAL VIABILITY = 95%*	INITIAL VIABILITY = 46%*	
%	%	%	%	%
0	3.1	60(7)††	25(3)††	41(10)††
5	3.7	63(5)	29(2)	41(10)
15	5.7	63(5)	27(3)	44(8)
25	7.3	80(1)	45	38(7)
45	9.6	83	39	54(6)
65	12.3	95	38	53(6)
85	20.1	91	21	47(5)
100§	37.3	49	2	53(4)

* Initial moisture content 9.0%.
† Initial moisture content 3.6%; initial germinability 38% + 9% hard seeds.
†† Figures in parentheses represent hard seeds.
§ Maintained with distilled water.

tables II and V and in figures 2B, 6, 7, and 8. Tables I, II and IV and figures 6 and 7 also present germinability data for many of these same seed lots. Essential information as to important differences in seeds used and in types of storage treatment is included with each table or figure. Seed moisture equilibria and germinability data are presented together in the same tables for convenience in comparisons, but are discussed separately in the following sections.

HYGROSCOPIC EQUILIBRIA OF NATURALLY AND ARTIFICIALLY CURED SEEDS

The hygroscopic equilibrium (table I and fig. 2A) for naturally dried lupine seeds stored for eight weeks over sulphuric acid solutions shows the characteristic moisture-exchange pattern throughout the humidity range

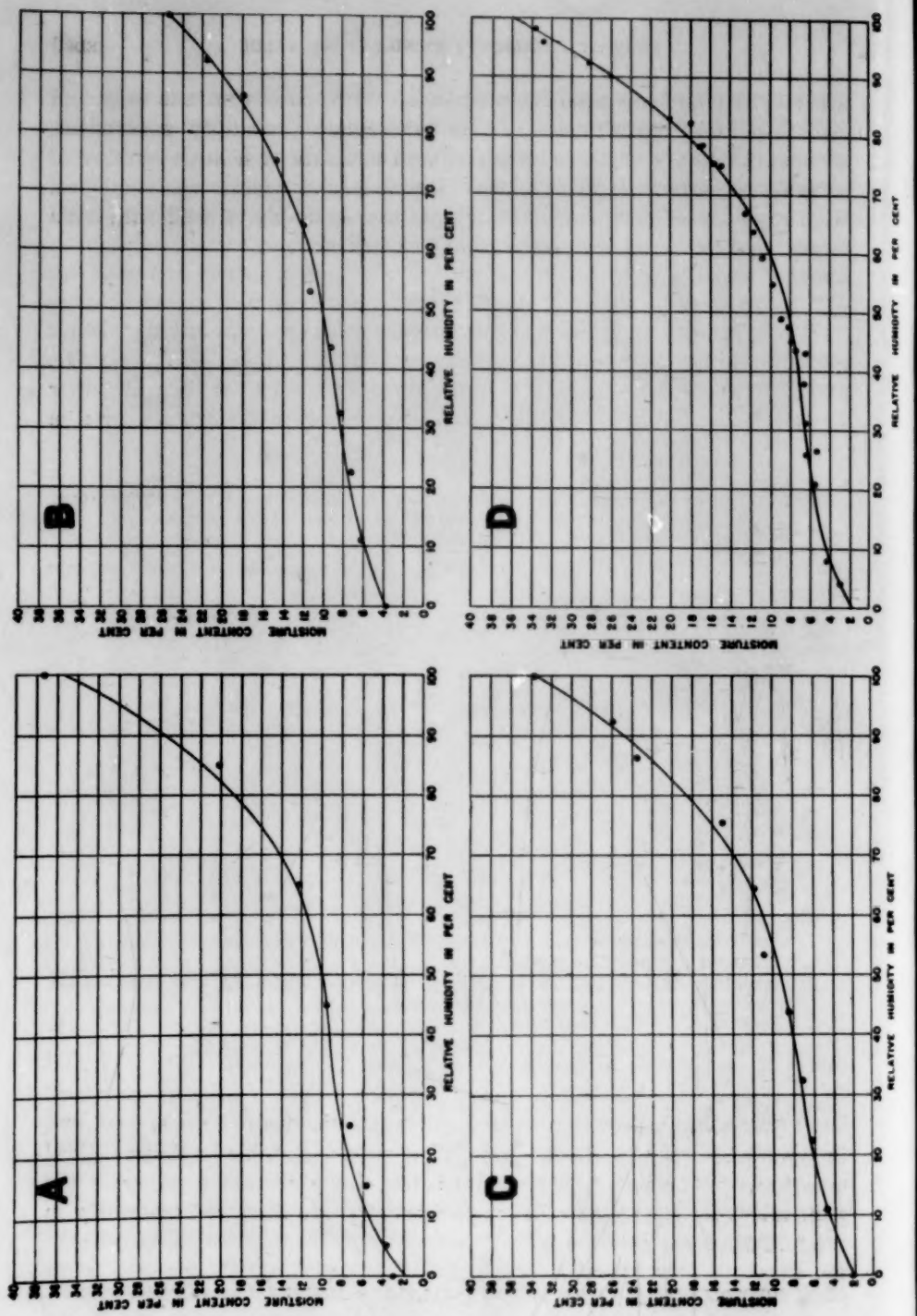

FIG. 2. Hygroscopic equilibria of blue lupine seeds. (A) Naturally dried seeds of 9.0% initial moisture content after storage for eight weeks—method of sulphuric acid solutions. (B) Naturally dried seeds of 12.1% initial moisture content after storage for six weeks—method of saturated salt solutions. (C) Naturally dried seeds of 12.1% initial moisture content after storage for eight weeks—method of saturated salt solutions. (D) Both naturally and artificially cured seeds of moisture contents ranging from 3.1 to 33.6%—electric hygrometer method.

that can be shown for lupine seeds by use of other methods. The moisture percentage of 37.3% for seeds held at 100% relative humidity is somewhat higher than has been found with similar treatment in some other experiments to be described below. These seeds showed a slight mold growth, and their relatively high moisture content was no doubt partly attributable to a decrease in seed dry matter due to fungal metabolism or to moisture held by the fungus mycelium itself.

TABLE II

MOISTURE PERCENTAGES AND GERMINABILITIES OF NATURALLY DRIED BLUE LUPINE SEEDS STORED FOR SIX WEEKS AND FOR EIGHT WEEKS AND OF ARTIFICIALLY DRIED BLUE LUPINE SEEDS STORED FOR FIVE WEEKS AT VARIOUS RELATIVE HUMIDITIES. METHOD OF SATURATED SALT SOLUTIONS

Relative humidity §	Naturally dried seeds*				Artificially dried seeds			
	Six weeks storage		Eight weeks storage		Drying Plant A†		Drying Plant B††	
	Moisture content	Viability	Moisture content	Viability	Moisture content	Viability	Moisture content	Viability
%	%	%	%	%	%	%	%	%
0	3.8	47	2.2	70	.....	.....	.....	.....
11.1	6.2	74	4.8	90	.....	.....	.....	.....
22.5	7.2	78	6.2	94	.....	.....	.....	.....
32.5	8.3	75	7.1	96	.....	.....	.....	.....
43.7	9.2	77	8.4	95	.....	.....	.....	.....
53.3	11.2	80	10.9	92	10.6	95	10.4	97
64.4	11.9	88	11.8	87	12.0	100	11.8	96
75.4	14.6	83	15.0	51	14.3	98	14.1	99
86.4	17.9	61	23.5	1	17.8	97	16.7	99
92.5	21.5	32	25.9	0	19.6	90	19.1	89
100	25.4	4	33.7	0	22.2	63	22.3	78

* Initial moisture content 12.1%; initial germinability 90%.
† Initial moisture content 10.8%; initial germinability 97%.
†† Initial moisture content 8.9%; initial germinability 93%.
§ Initial 0% humidity with concentrated sulphuric acid; 100% humidity maintained with distilled water; humidities of 11.1 to 92.5, inclusive, with saturated solutions of salts listed in text.

Workers using other seeds (**4**, **9**, **10**, **11**, **12**, **15**) found that hygroscopic equilibrium between seeds and air was reached in five to six weeks or less. Preliminary experiments with blue lupine seeds stored over water (100% relative humidity) and over twice-replaced concentrated sulphuric acid (approximately 0% initial relative humidity) had shown seed wet weight changes with each weekly weighing until the seventh week but negligible changes from the seventh week onward. It was therefore assumed in all subsequent experiments that an 8-week storage period would suffice for equilibria to be reached. It is possible that this assumption was erroneous, especially at 100% relative humidity where wet weight increases are

partially offset by dry weight decreases caused by microbial activity. However, it is believed that moisture changes after the eighth week are of no practical significance except where obvious seed deterioration occurs. The experiment whose results are presented in table II and figure 2 (B, C) was designed to show the differences between the 6-week and 8-week storage periods in effects on seed-air moisture exchanges throughout the relative humidity range. From the preliminary studies and from these data, it was concluded that naturally dried blue lupine seed apparently requires seven to eight weeks to attain equilibrium with air of high and low relative humidities but not more than six weeks with air of intermediate humidities. Artificially cured lupine seeds in contact with air of about 65% relative humidity may reach equilibrium in as short a period as three weeks, as shown below. Data in table II and figure 2 (B, C) show that blue lupine seeds of 12.1% initial moisture content stored at a relative humidity initially at nearly

TABLE III

RELATIVE HUMIDITY PERCENTAGES PRODUCED AT EQUILIBRIUM IN CONFINED AIR BY BLUE LUPINE SEEDS OF DIFFERENT MOISTURE CONTENTS. ELECTRIC HYGROMETER METHOD

MOISTURE CONTENT OF SEEDS	RELATIVE HUMIDITY	MOISTURE CONTENT OF SEEDS	RELATIVE HUMIDITY	MOISTURE CONTENT OF SEEDS	RELATIVE HUMIDITY
%	%	%	%	%	%
3.1	4.0	7.9	45.0	16.7	78.5
4.4	8.0	8.2	47.5	17.0	78.5
5.4	26.5	8.9	49.0	18.0	82.3
5.5	20.8	9.8	54.7	20.0	83.2
6.4	31.2	10.9	59.3	28.1	92.5
6.5	26.0	11.7	63.8	32.8	96.0
6.5	43.1	12.5	66.9	33.6	98.1
6.6	38.0	15.0	75.0	.....	.....
7.4	43.5	15.6	75.5	.....	.....

0% were dried to 3.8% in six weeks and to 2.2% in eight weeks, while if stored at 100% relative humidity, they increased in moisture content by absorption up to 25.4% in six weeks and up to 33.7% in eight weeks. The difference in the moisture-exchange relationship is clearly shown by the slopes of the curves in figure 2 (B, C).

The electric hygrometer was used in a series of determinations of the moisture relations between air and lupine seeds of various moisture contents. Excellent agreement was obtained between the curve plotted for data determined by the use of the hygrometer (table III and fig. 2D) and the curves representing hygroscopic equilibria found in the storage tests for 8-week periods in which either sulphuric acid (fig. 2A) or saturated salt solutions (fig. 2C) were used. The practical advantage of securing equilibria data in 24 to 72 hours by means of the hygrometer over securing them by eight weeks' storage with humidity-control solutions is obvious. The hygrometer has the disadvantage of requiring seeds of a complete range of moisture

contents. Some of these moisture percentages are below those held in equilibrium with the normal atmosphere and must be produced by supplemental pre-drying.

The equilibrium moistures reached by naturally cured blue lupine seeds in the test simulating commercial storage conditions (table IV and fig. 3) show that the final seed moisture content is not materially governed by the initial moisture content but is primarily a function of the relative humidity at which the seeds are stored. The apparent tendency shown by data in table IV for seeds of the higher initial moisture contents to have higher final moisture percentages after storage at the same initial relative humid-

TABLE IV

MOISTURE PERCENTAGES AND GERMINABILITIES OF NATURALLY DRIED BLUE LUPINE SEEDS OF DIFFERENT INITIAL MOISTURE CONTENTS AFTER STORAGE FOR SIX MONTHS AT VARIOUS RELATIVE HUMIDITIES. METHOD OF SULPHURIC ACID SOLUTIONS

Initial moisture level group	Relative humidity		Moisture content		Germinability	
	Initial	Final	Initial	Final	Initial	Final*
	%	%	%	%	%	%
Excessive	90	87	20.5	19.5	49	0
	80	77	20.8	15.1	66	39
	70	68	20.5	13.5	45	85
	60	58	20.8	11.6	71	94
High	90	85	15.8	17.8	66	2
	80	75	15.8	14.8	73	96
	70	68	15.7	12.9	78	99
	60	56	16.1	11.1	58	96
Average	90	83	13.6	16.6	74	47
	80	70	13.6	13.6	77	98
	70	54	13.7	10.9	66	97
	60	44	13.6	10.6	73	94
Low	90	78	10.0	17.7	74	30
	80†	.....	.....	.....	.....	.....
	70	68	10.1	12.5	84	97
	60	58	10.1	11.1	78	98

* Seeds of all moisture contents stored at 100% r.h. were obviously non-viable.
† Insufficient seed supply to store at this humidity.

ities is largely explained by the fact that the relative humidities of the confined air in these cases had been increased by the amount of water vapor evaporated from the seeds. If all of the final relative humidity data of table IV, disregarding original seed moisture contents, are rearranged in an increasing or decreasing order of magnitude, the final seed moisture contents follow essentially the same order. However, in those cases in which the final relative humidities for different storage samples were the same, the final seed moisture contents were in the same order as the initial percentages. For example, with the initial storage relative humidities of 70%, which decreased to 68% at the end of six months, the final seed moisture contents were 13.5% for seeds initially holding 20.5% water, 12.9% for seeds initially

at 15.7%, and 12.5% for seeds initially at 10.1%. Whether further storage beyond six months would have equalized these differences is questionable.

Presented in tables II and V and figures 4 and 5 are results of a test set up to determine whether experiment would support the opinion expressed by some seedsmen that blue lupine seeds artificially dried to about 10% moisture content can thereafter be exposed to high humidities without serious damage. In figure 4 are shown the results only for the absorption of moisture by artificially cured lupine seeds of an initial moisture content

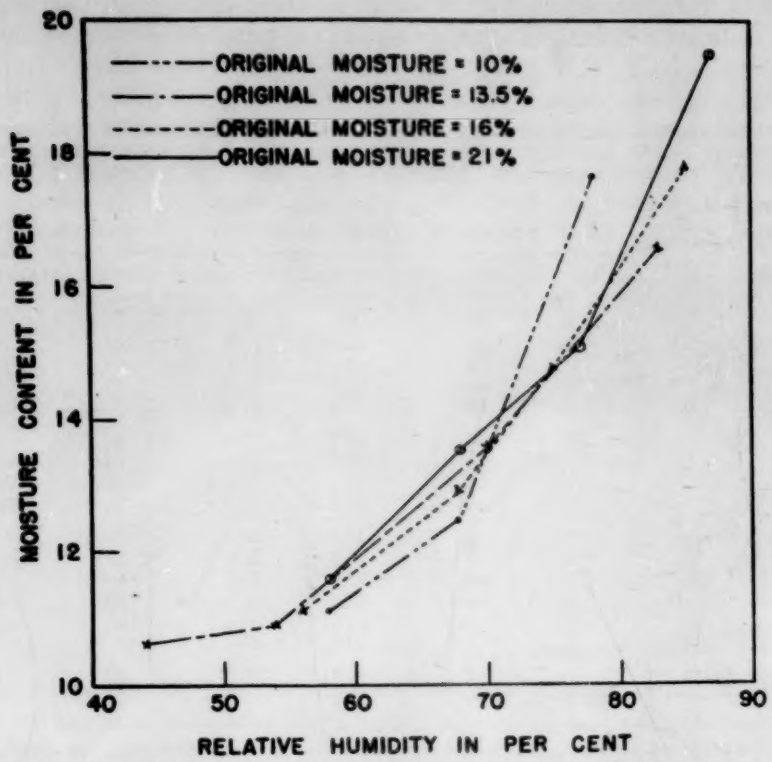

FIG. 3. Equilibrium moisture percentages of naturally dried blue lupine seeds of different initial moisture contents after storage for six months at various relative humidities. Method of sulphuric acid solutions.

of 10.8%, but curves plotted for artificially dried seeds holding 8.9% moisture when first exposed to the different humidities were quite similar. However, figure 5 illustrates the lasting effect of a difference in initial moisture content upon the rate at which lupine seeds reach equilibrium with the moisture of the air. Although stored at the same humidities, the seeds of 8.9 and 10.8% initial moisture contents had not reached like moisture percentages after five weeks. As shown above (table IV), seeds initially differing in moisture percentages by as much as 4.8% had not reached the same moisture content after storage at the same humidity for six months. In

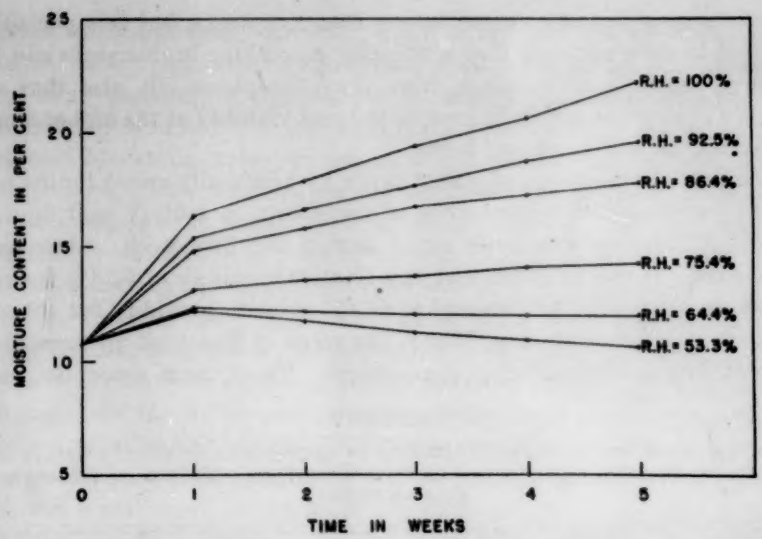

FIG. 4. Percentages of moisture held by artificially dried blue lupine seeds of 10.8% initial moisture content at weekly intervals during storage at various relative humidities. Method of saturated salt solutions.

the 6-month storage test, the use of sulphuric acid solutions for humidity control had prevented the maintenance of constant relative humidity throughout storage, but in the 5-week test using saturated salt solutions at constant temperature, this difficulty was avoided. Time limitations pre-

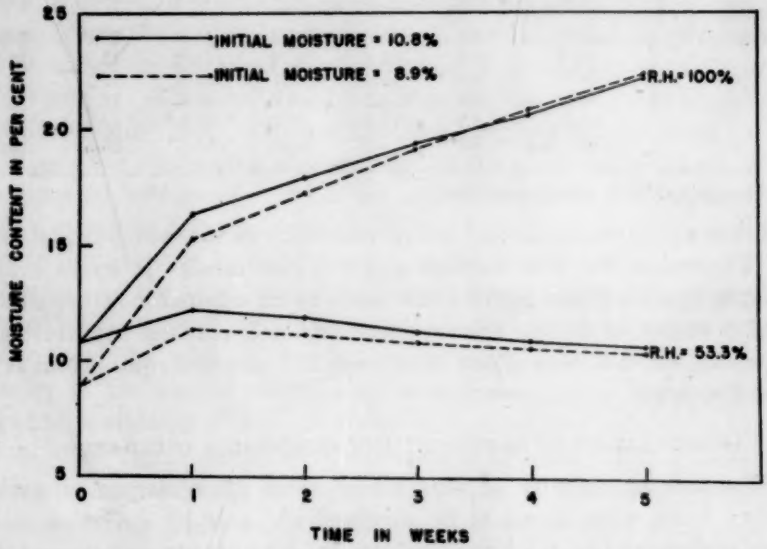

FIG. 5. Percentages of moisture held by artificially dried blue lupine seeds of 10.8 and 8.9% initial moisture contents at weekly intervals during storage at 53.3 and 100% relative humidities. Method of saturated salt solutions.

vented running this experiment longer than five weeks, but this period was sufficient to show not only that artificially cured blue lupine seeds can take up large amounts of moisture from the atmosphere but also that such moisture absorption results in greatly reduced viability at the higher storage humidities, as will be shown below.

The rate of absorption of water vapor by artificially cured lupine seeds held at various relative humidities is also shown in table V and figures 4 and 5. Absorption was quite rapid during the first week, subsequently leveling off. It will be noted that at a relative humidity of 53.3% for seeds from both commercial sources and at 64.4% relative humidity for the seeds of initial moisture content of 10.8%, the seeds at first took up moisture in excess of their final equilibrium percentages. This type of absorption seems

TABLE V

PERCENTAGE MOISTURE AT WEEKLY INTERVALS IN ARTIFICIALLY DRIED BLUE LUPINE SEEDS HELD FOR FIVE WEEKS AT VARIOUS RELATIVE HUMIDITIES. METHOD OF SATURATED SALT SOLUTIONS

SOURCE OF SEED	RELATIVE HUMIDITY	MOISTURE CONTENT (WET BASIS)					
		INITIAL	1 WEEK	2 WEEKS	3 WEEKS	4 WEEKS	5 WEEKS
	%	%	%	%	%	%	%
Commercial drying plant A	53.3	10.8	12.2	11.8	11.3	10.9	10.6
	64.4	10.8	12.3	12.1	12.0	12.0	12.0
	75.4	10.8	13.1	13.5	13.8	14.1	14.3
	86.4	10.8	14.8	15.8	16.7	17.2	17.8
	92.5	10.8	15.4	16.6	17.7	18.8	19.6
	100 *	10.8	16.3	17.9	19.4	20.6	22.2
Commercial drying plant B	53.3	8.9	11.3	11.1	10.8	10.6	10.4
	64.4	8.9	11.4	11.4	11.6	11.7	11.8
	75.4	8.9	12.1	12.7	13.3	13.7	14.1
	86.4	8.9	13.5	14.5	15.4	16.1	16.7
	92.5	8.9	14.2	15.7	17.1	18.1	19.1
	100 *	8.9	15.2	17.3	19.2	20.8	22.3

* Maintained with distilled water.

to indicate a progressive decline in hygroscopicity of the seed colloidal material. The reason for this decrease was not discovered. It seems evident from data in table V and figure 4 that seeds of an original moisture content of 10.8% stored at 64.4% relative humidity had reached equilibrium in three weeks, but that none of the other seeds had attained equilibrium at the end of five weeks.

GERMINABILITY OF NATURALLY AND ARTIFICIALLY CURED SEEDS

Differences in viability of blue lupine seeds after storage at various humidity levels were shown to be governed not only by variation in the storage periods and in the humidities at which the seeds were stored, but also by such pre-storage factors as degree of maturity, initial moisture content, initial viability, and nature of the drying process.

Seeds which had been chemically dried to the very low moisture content of 3.6% (table I) showed no significant viability differences in treatment effects when exposed for eight weeks to relative humidities ranging from approximately zero to 100%, although the number of seeds subsequently incapable of absorbing water decreased as the relative humidity of storage was increased. The chemical pre-drying treatment of these seeds before they were exposed to humidity treatments, however, had reduced their germinability from 85% at time of harvest to 38% (plus 9% hard seeds) while other seeds of the same original lot stored under laboratory conditions showed no viability loss. In all other cases where seeds were stored at extremely low humidity levels (tables I and II), this same decline in germinability was evident, regardless of initial moisture content or viability. In some instances, the differences in germinability were not large enough for statistical significance, but the consistently observed reduction in viability with low storage humidities throughout all tests leaves little doubt that the effect was a real one. It cannot of course be attributed to greater fungal activity at low than at medium or high humidities. Hence, it appears that extreme drying of the seeds has some permanently detrimental effect that is not necessarily connected with subsequent inability of the seeds to absorb sufficient water to support the germination processes.

Significant differences in germinability were shown (table I) between air-dry seeds of high and of low initial viability, depending upon the storage humidity to which they were exposed. Seeds of high initial viability (95%) maintained this same percentage of germinability at a relative humidity of 65%, with decreases in viability as relative humidity was either increased or decreased from 65%. The same sort of decline from a peak in germinability with increased or decreased relative humidity was shown in seeds of low initial viability (46%), but the highest viability was here found at a relative humidity of only 25%. These differences are perhaps too slight to have significance for the number of seeds used for the germination counts. They are supported, however, by similar peaks in germinability in all experiments where a complete range of humidities was used.

Blue lupine seeds stored for eight weeks showed greater injury than those stored at the same humidities for six weeks (table II), as would be expected. The peak of germination in the 6-week storage test was at 64.4% relative humidity, while it occurred in the range of 22.5 to 43.7% relative humidity in the 8-week period, emphasizing the greater damage to seeds even of high viability if they are stored at high relative humidities for long periods. The higher germination percentages of seeds stored at humidities ranging from 0 to 53.3% for eight weeks than of those held at the same humidities for six weeks can possibly be attributed to maturation processes, since a similar progressive increase in germinability of seeds of the same original source was observed in some other experiments as time passed, whenever storage conditions were favorable.

The interactions between initial seed moisture and quality, storage humidity, and length of storage are well illustrated in figure 6 plotted from data of tables I and II. The relative heights of the five curves and the positions of their peaks in relation to atmospheric humidity levels graphically emphasize the importance of both seed and environmental factors in determining viability changes during storage.

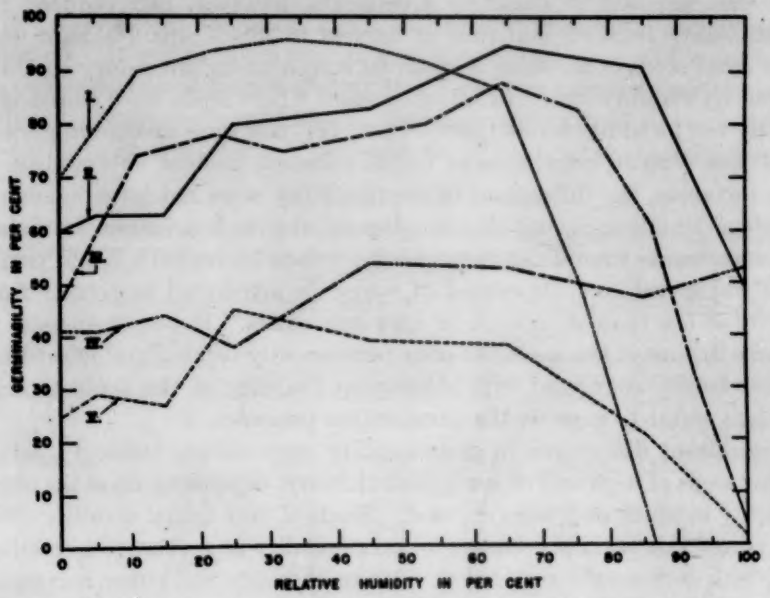

FIG. 6. Effect of storage at different relative humidities on germinability of blue lupine seeds of different initial viabilities and moisture contents:

Curve No.	Initial moisture content, %	Initial viability, %	Length of storage, weeks
I	12.1	90	8
II	9.0	95	8
III	12.1	90	6
IV	3.6	38	8
V	9.0	46	8

Germinabilities of the lupine seeds at the start of the test simulating commercial storage (table IV) were extremely variable as well as unexpectedly low. This was believed to be due to the fact that many seeds were immature at the time of harvest, but may also indicate that the conditions which are most favorable for germination of mature lupine seeds are not optimum for immature seeds. Data for final germinabilities (see also fig. 7) were much more consistent. In addition to the results given in table IV and figure 7, seeds of the same four initial moisture levels were also stored at 100% relative humidity. Seeds held at this humidity showed such deterioration as to be obviously non-germinable, and germination tests were not run. Seeds stored at 90% relative humidity also were greatly damaged

(table IV and fig. 7), regardless of initial moisture, although seeds of the 13.6 and 10.0% initial moisture contents showed significantly less injury than did those of greater moisture.

Data in table IV indicate that blue lupine seeds of 20% moisture can be stored without damage for long periods only at relative humidities less than 70%, and probably less than 65%. Seeds of up to about 16% moisture

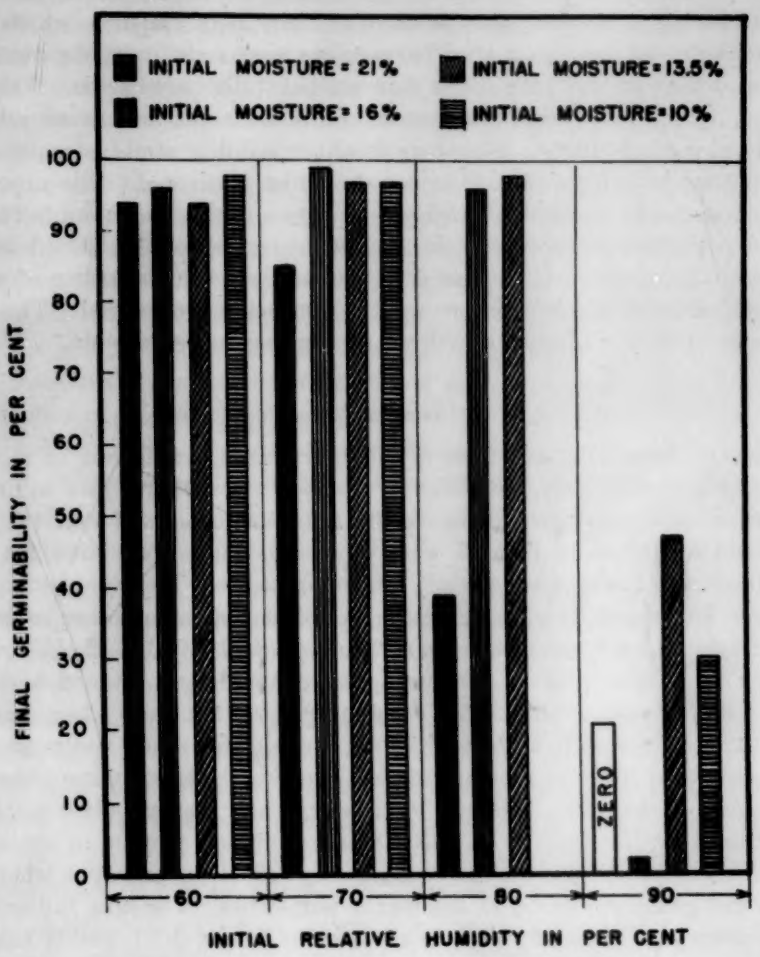

FIG. 7. Germinabilities of naturally dried blue lupine seeds of different initial moisture contents after storage for six months at various relative humidities.

content apparently can be stored without injury at humidities up to 75%. As was shown in table I, high quality naturally cured seeds of less than 10% moisture content can be stored without great damage for eight weeks at relative humidities as high as 85%. However, due to an insufficient quantity of seeds of 10% moisture content for the complete series (table IV),

it was not determined whether seeds of this moisture content can be held at 80% relative humidity for as long as 6 months.

The supposition of some seedsmen that blue lupine seeds artificially dried to about 10% moisture content subsequently do not absorb much water even from air of high relative humidity was shown to be erroneous (table V and figs. 4 and 5). Germinability data taken simultaneously with moisture absorption data for these same artificially cured seeds indicate strongly, however, that seeds of initially high viability, which have been artificially dried soon after harvest, are more resistant to deterioration under storage at high humidities than are naturally cured seeds. Table II shows high or relatively high germinabilities for seeds held at all relative humidities except 100%. How long this high viability would be maintained under storage at high humidities remains to be discovered. The nature of this resistance to damage is possibly related to a lasting reduction in fungus spore populations resulting from the drying process. It should be investigated, as it seems to have no direct connection with absorption of water by the seeds in which moisture uptake appears to be normal. The seed cleaning done in conjunction with the drying may be responsible.

Discussion

Results from determinations of the hygroscopic equilibrium of blue lupine seeds produced by the different methods of sulphuric acid solutions, saturated salt solutions and an electric hygrometer showed that the data obtained by the three methods were in essential agreement provided that equilibrium actually was reached. Blue lupine seeds seem to attain equilibrium with humidity of the air more slowly than do many other seeds and grains for which results have been reported (**4, 9, 10, 11, 12, 15**). This slower rate of absorption of moisture in the vapor phase is coupled, however, with a higher total uptake than is found in many other seeds. Lupine seeds also have exceptionally high imbibitional capacity for liquid water, increasing as much as 165% in moisture content (dry weight basis) when immersed in water. It has been assumed by RAMSTAD and GEDDES (**14**), although questioned by LARMOUR *et al.* (**12**), that a high ash content in seeds and grain is responsible for an increased absorption of water vapor when the seeds and grain are stored at the higher humidities. The blue lupine seed is, however, not unusually high in ash content (about 3%), and if ash elements are accountable for increasing hygroscopicity at high storage humidities in some seeds, this explanation apparently does not hold for blue lupine seeds. The unusual moisture sorption properties of blue lupine seeds seem to be due primarily to colloidal components of the seeds.

Most hygroscopic equilibria studies with other seeds have been limited to the range of humidities (about 30 to 90% relative humidity) most likely to be encountered under the usual storage conditions. Those experiments, therefore, have failed to reveal the difference in the type of moisture ex-

change between seeds and the water vapor of the air at humidities below about 50%, throughout which range the seeds lose moisture to the air rather than gain moisture from the air. This difference in the desorption end of the moisture equilibrium curve is particularly obvious when experimental humidities approaching zero are employed. In blue lupine seed, the rate of increase in the amount of water held by the seeds decreases as the relative humidity increases up to about 40%. Hygroscopic equilibria studies with other seeds (**9, 12, 14, 15**) have not demonstrated a similar moisture-retention pattern for various reasons. When the lowest humidities were omitted from experiments, the absence of points for plotting the lower end of the curve made an interpretation of its rate and direction of curvature difficult. Hence the data often were presented graphically as parabolic segments, whereas in some instances, mathematical treatment of the data would have made clear the sigmoid nature of the curve. The reversal of the rate of increase in water held by the seeds with increasing storage humidity was clearly shown in these tests with blue lupine seed. It was also demonstrated by KARON and ADAMS (**10**) for cottonseed and cottonseed products, and by KARON and HILLERY (**11**) for peanuts. Regression equations calculated from data of LARMOUR *et al.* (**12**) for soybeans, flaxseed, and sunflower seed can be shown to follow the same pattern, as can those computed from data of COLEMAN and FELLOWS (**4**) for cereal grains and flaxseed. Coleman and Fellows concluded that a parabola most correctly portrayed their data, although the curves actually plotted for their results were not parabolic.

In these cases, as well as in the blue lupine experiments here reported, computation of regression equations for the best-fitting curves by the method of least squares (**16**) shows that the experimental results are excellently represented by curves of the third degree ($Y = a + bX + cX^2 + dX^3$, where Y = seed moisture content and X = relative humidity). Parabolic equations do not agree well with the data.

The regression equations for the hygroscopic equilibria of blue lupine seeds held for eight weeks over sulphuric acid solutions, for six weeks and for eight weeks over saturated solutions of various salts, and for equilibria as determined by an electric hygrometer are presented below:

H_2SO_4 solutions	$-Y = 1.868 + .4612X - .01067X^2 + .00009401X^3$	(fig. 2A)
Saturated salt solutions, six weeks	$-Y = 3.815 + .2468X - .004578X^2 + .00004265X^3$	(fig. 2B)
Saturated salt solutions, eight weeks	$-Y = 2.079 + .3146X - .006898X^2 + .00006892X^3$	(fig. 2C)
Electric hygrometer	$-Y = 1.862 + .3248X - .008101X^2 + .00008256X^3$	(fig. 2D)

It will be noted that the equation for the 6-week exposure of blue lupine seeds to saturated salt solutions differs materially from the other three equations. This is true because equilibrium was not actually reached in the 6-week period. For purposes of better comparison with the other three equations, the equation for the electric hygrometer determinations was calculated by plotting seed moisture content (Y) against relative humidity (X) as determined by the hygrometer, although it is perhaps more correct to consider relative humidity rather than seed moisture content as the dependent variable in this instance.

The four curves of figure 2 are superposed in figure 8 for advantage in

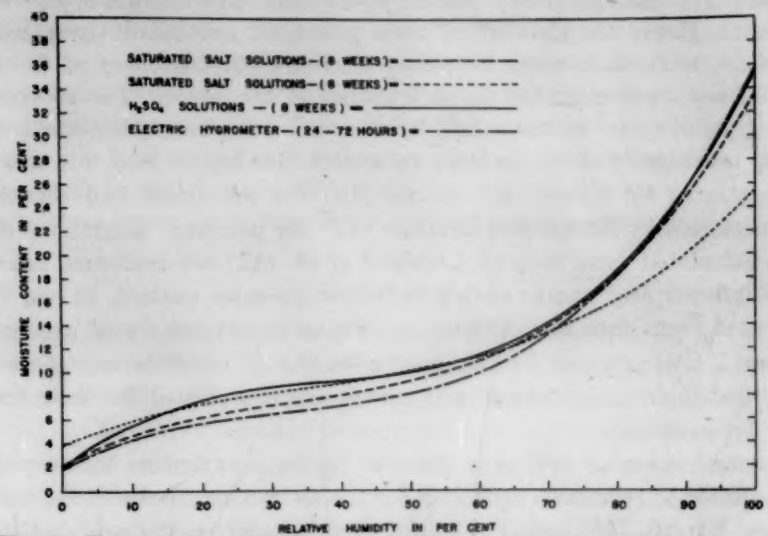

FIG. 8. Hygroscopic equilibria curves for blue lupine seeds determined by methods of saturated salt solutions (6- and 8-week exposures), sulphuric acid solutions (8-week exposure), and electric hygrometer (24- to 72-hour exposure).

comparison. It is evident that moisture equilibrium between seeds and air was not reached in six weeks at either the high or the low humidities. The divergence of the central portion of the curve representing the equilibrium obtained by use of the sulphuric acid solution method is no doubt explained by the fact that initial relative humidities were used for plotting purposes, whereas the final equilibrium humidities, if determined, would in each case have been higher in this moisture range because of the increased moisture in the air resulting from evolution of water vapor from the seeds. Similarly inaccurate data in this lower moisture range may be expected whenever sulphuric acid solutions are used as the means of obtaining relative humidities. It is known that the relative humidity of enclosed air over a sulphuric acid solution decreases or increases if in contact with materials having initial moistures lower or higher, respectively, than those held by the materials after equilibrium has been reached. With saturated salt solutions, on the

other hand, water may be lost to dry materials or gained from wet materials with which the air over the solution is in contact, but the relative humidity of the confined air at constant temperature and pressure is unchanged as long as an excess of salt remains.

The speed and convenience of the electric hygrometer in measuring hygroscopic equilibria recommend its use for this determination. Data obtained in 24 to 72 hours with the hygrometer were in good agreement with those found by the saturated salt solution method in eight weeks. The instrument is less well adapted to use at the lower humidity ranges, however, where it is necessary to pre-dry the seeds to moisture contents below those held by seeds in equilibrium with normal air. Whether the slight downward divergence of the hygrometer curve (fig. 8) from the salt solution method curve in the lower moisture ranges and its slight upward divergence at humidities above 85% are characteristic of the instrument remains to be investigated.

The more harmful effect of high storage humidity (table I and fig. 6) upon seeds of initially low germinability (46%) than upon seeds of initially high germinability (95%) supports the inference of GILMAN and SEMENIUK (**6**) and others that, since the principal mold populations found on seeds and grains are saprophytes, further deterioration caused by microorganisms during storage is greatest in seeds initially low in vitality. Once they are established on these low quality seeds, the saprobes are better able through the action of secreted toxins to attack the good seeds with which they are in contact. Seeds already containing a large proportion of non-living material, therefore, are subject to continuing spoilage by saprophytic organisms at humidities which are too low to favor mold development on seeds having high initial viabilities. The predominance of saprobic populations in deteriorated seeds does not preclude the possibility of action of parasitic organisms in extensive seed damage, especially where respiratory activity of the saprobes has improved growth conditions for all molds (**6**).

The effect of decreasing oxygen and increasing carbon dioxide supply upon viability in blue lupine seeds in sealed containers of the type used in these experiments is not known. In prolonged storage, this change in the proportion of these gases would depress the respiration rate, but whether carbon dioxide would accumulate in toxic concentrations, particularly in the seeds of higher moisture contents, is uncertain. BARTON (**1, 3**) has concluded that sealed storage favors the maintenance of viability of seeds, except at high seed moisture contents. However, these benefits were most marked at temperatures of 5° C or lower, and with seeds stored at room temperatures, she found that sealing the containers did not prevent extensive loss of viability in dandelion and aster (**1**), or provide any advantage over open storage in cinchona (**3**). Barton attributes the beneficial effects of open storage of seeds of high initial moisture content to a drying of the seeds, rather than to avoidance of any supposed harmful effect of accumulated

carbon dioxide that might occur in sealed storage. She also considers that the decrease in germinability of seeds held in containers which are periodically opened is a result of the consequent fluctuations in humidity of the air to which they are exposed (**2, 3**). Barton's experiments ran over much longer periods than those on blue lupine reported here.

In these experiments with lupine seeds, the artificially cured seeds (table V) were re-exposed to normal air at weekly intervals when each weighing was made to determine moisture exchanges between seeds and air. In all other tests, the seed containers remained closed until the end of the experiments. Because of differences in initial viability and in length of storage, it is difficult to appraise the possible effects of carbon dioxide accumulation upon eventual germinability. It seems probable, however, that oxygen deficiency and carbon dioxide toxicity were negligible factors in seed deterioration under the conditions of these experiments.

Summary

1. The relationship at equilibrium between seed moisture percentage and relative humidity of the enveloping air was determined for blue lupine seeds of moisture contents ranging from 2.2% to 37.3% and for relative humidities ranging from approximately zero to 100%. The equilibrium point for any particular combination of seed moisture content and relative humidity was not significantly different whether determined by means of an electric hygrometer or by the use of humidifying and drying solutions prepared with sulphuric acid or saturated with selected salts.

2. Moisture contents of seeds held over the solutions producing the various relative humidities did not reach equilibrium with the highest or lowest experimental humidities in less than eight weeks. Moisture of seeds held at about 65% relative humidity attained equilibrium with moisture of the air in three weeks.

3. The electric hygrometer was found to be a convenient, rapid, and accurate means of determining the hygroscopic equilibrium of seeds. The equilibrium relative humidities shown by this instrument to be reached in 24 to 72 hours in confined air in contact with seeds of various moisture contents were in essential agreement with the humidities found to produce these same moisture contents in seeds after prolonged storage.

4. The rate and total amount of moisture absorption or loss did not differ significantly between blue lupine seeds artificially dried with heated air and seeds naturally cured in the field.

5. At relative humidities up to about 40%, the rate of increase in seed moisture content decelerated with increase in relative humidity, and thereafter accelerated with each increment of increase in humidity. The equation that best represents this seed moisture-relative humidity relationship is a third degree type, rather than the parabolic equation corresponding to the curves which usually have been plotted to present hygroscopic equilibria data with other seeds.

6. The relative humidities at which blue lupine seeds can be stored safely depend upon such seed qualities as proportion of cracked or non-viable seeds, moisture content, nature of the curing process and age, and such storage factors as temperature and duration of storage. Under the conditions of these experiments, initial seed viability was the property of greatest importance in determining safe storage humidities.

7. In 8-week storage tests, naturally dried seeds of initial germinability of 46% decreased in viability if stored at relative humidities above 25%, while naturally dried seeds of viabilities of 90% or higher showed little or no reduction in germinability if stored at about 65% relative humidity.

8. Artificially cured seeds showed no reduction in viability when stored for five weeks at relative humidities as high as 86.4% and a reduction of less than 10% in viability when held for the same period at 92.5% relative humidity.

9. Regardless of their moisture contents at the beginning of storage, naturally cured lupine seeds held in closed containers for six months showed complete or almost complete loss of viability if their final moisture content was above 15% or if the relative humidity of the air of the storage container at the end of the period was 85% or higher. Viability losses of 50 to 100% were shown by seeds of initial moisture content of 21% held at all relative humidities of 70% or higher and by seeds initially holding as little as 13.6% moisture if stored at relative humidities of 83% or higher.

10. Blue lupine seeds during storage showed consistent losses in viability at the lowest as well as at the highest experimental humidities. Germinability declined with both increases and decreases in storage humidity from peak germination counts at about 25% relative humidity for low quality seeds and at 65% relative humidity or higher for seeds of high initial viability. Losses in viability at low storage humidities had no apparent relation to subsequent inability of the seeds to absorb normal amounts of water, since few hard seeds were produced except during prolonged storage at the lowest humidities.

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THE RELATION OF THE CHARACTERISTICS AND YIELD OF WAX TO PLANT AGE

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(WITH TWO FIGURES)

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Introduction

Plants have been used as a source of wax for centuries, and the search for plant species that produce more wax of a better quality has been in progress for as many years. It has been recognized only for the past few decades, however, that various factors other than plant species, such as environment, genetic make-up of the plant, and maturity of the plant, may affect the quality and quantity of wax exudate. MCNAIR (**11**) has shown that there is a definite relation between the properties of plant waxes and climate; and CONRAD and NEELY (**2**) in a study of cotton showed that an hereditary factor controls the quantity of lint wax. Only fragmentary evidence, however, has been presented to show that plant maturity may affect the deposition of wax on the plant surfaces. HOWES (**5**) observed that the yield of wax from the carnauba palm, candelilla, and fruits of bay-berry varied in some way with age of the plant organs. DAHLGREN (**3**) studied the yield of carnauba wax from unexpanded and expanded leaves and concluded that under some conditions expanded or old leaves yield more wax per leaf than unexpanded or young leaves. Studies by SAHAI and CHIBNALL (**13**) on the wax of Brussels sprout, and JORDAN and CHIBNALL (**8**) on the wax of the runner bean, have shown that wax is synthesized continually throughout the life of the plant, with the formation of wax occurring not just at the growing tips but in all the plant organs except the roots. It is apparent, therefore, that the synthesis and yield of wax are related to plant age, and that the relationship might vary greatly in some cases. It has been the purpose of the present study to observe the relation of the yield and the physical and chemical characteristics of wax to plant maturity.

Materials and methods

On the basis of time of collection and external structural characteristics, two ages (young and old) of leaves or stems of *Phragmites communis* (Gramineae); *Acacia greggii, Acacia vernicosa, Acacia constricta, Olneya tesota* (Leguminosae); *Asclepias linaria* (Asclepiadaceae); *Beloperone californica* (Acanthaceae); staminate and pistillate plants of *Baccharis sarothroides, Aplopappus tenuisectus,* and *Aplopappus laricifolius* (Compositae) were collected for study from their natural habitats. These species

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are all indigenous to southern Arizona, and are representative of wide ranges of environmental conditions of altitude, rainfall, incident light, and edaphic factors. Several ages of leaves of *Sorghum halepense* (Gramineae) and pistillate *Phoenix dactylifera* (Palmaceae) were also studied. Eight ages of plant material of *S. halepense* were distinguished by their position on the culm. The inflorescence and seven ages of leaves and nodes were collected separately, progressing from the inflorescence and youngest node and leaf at the top to the oldest node and leaf at the base of the culm. The time interval between each age of node and leaf was approximately five days. From their position on the tree, leaves of *Phoenix dactylifera* of 0, 3, 12, 18, 24, and 36 months of age were distinguished for study.

Several pounds of each age of plant material were collected and air-dried. The residual moisture after drying was 5% or less. A modification of the method of CHIBNALL *et al.* (1) was used to extract and separate fractions referred to as the wax and non-wax fractions. The air-dried plant material was refluxed for two hours with petroleum ether (B.P. 30° to 60° C). Decantation of the extract followed by several rinses of the plant material with fresh hot solvent completed the extraction. The extract was reduced to 250 ml. by distillation of the excess solvent. The wax was precipitated as an acetone-insoluble fraction by the addition of 500 ml. of acetone. The acetone-soluble fatty substances, or non-wax fraction, were recovered by evaporation of the solvent after removal by filtration of the so-called wax fraction. The per cent. yield of both wax and non-wax fractions was calculated on the basis of the weight of the dry plant material extracted.

The wax fractions were characterized physically by the determination of the drop point melting point (12), with the exception of a few cases in which the capillary rod drop melting point (9) was determined because only minute quantities of the wax samples were available. Relative hardness, color, and odor of the waxes were also noted.

Chemical characterization of the wax and non-wax fractions was accomplished by determining the acid, saponification, ester, and Wijs iodine numbers (6). These tests give, respectively, the amount of free acid, saponifiable matter, and esters present in, and the degree of unsaturation of the lipid fractions from the different ages of plant material.

At the beginning of the study an attempt was made to correlate the variations of wax yield and characteristics with changes in the carbohydrate content of the different ages of plant. Determination by the method of HASSID (4) of the milligrams per gram of the different ages of leaves and nodes of *Sorghum halepense* of total sugars, reducing sugars, sucrose, and starches and dextrins failed to yield any data of apparent significance. This line of study was not continued.

Because the wax yielded from a plant is generally believed to be derived from the cuticle, the relationship between wax yield and cuticle thick-

ness was studied. A small sample of each age of plant material studied was killed and fixed in formalin-aceto-alcohol and then run up through the tertiary butyl alcohol and paraffin series according to JOHANSEN (**7**). After sectioning at ten microns and staining, the cuticle thickness was measured in microns.

Results and discussion

The relationship of wax yield to plant age varied with different species, and on a weight per cent. basis increased in some cases and decreased in others. Nevertheless, in most cases (with the exception of *Acacia greggii, Acacia constricta, Aplopappus laricifolius,* and *Phoenix dactylifera*) this yield change with age was found to be directly related to cuticle thickness (tables I and II). This indicates that the main source of wax is the cuticle, and that only minor amounts of wax would be found in the protoplasm. According to earlier studies on Brussels sprout (**13**) and the runner bean (**8**), wax is steadily synthesized as the plant matures and is not catabolized. If this behavior is considered general, then the decrease with age of wax yield in several cases of the present study was only an apparent decrease. It is probable that by abrasive action of rain, wind, and dust on the cuticle, or by flaking and sloughing of the cuticle as the plant matured, the cuticle in these cases decreased in thickness and therefore the yield of wax decreased. Very marked increase of the dry weight of the plants with age may also have caused an apparent decrease of wax yield. In general it may be concluded that the total wax content of a plant increases with plant maturity; and in species requiring many years to mature, wide variation of yield may be found between young and old parts.

The yields of the non-wax extracts also show considerable variance with age, but there is no indication from yields that wax was synthesized at the expense of the non-waxes.

The results in tables I and II and figures 1 and 2 show that the wax melting point usually remained unchanged or it increased slightly with age. It also will be noted that the iodine number of the wax fraction usually decreased with age. A comparison of the two characteristics shows that in *Acacia greggii, Acacia constricta, Beloperone californica, Asclepias linaria,* and *Sorghum halepense* an increase of the melting point with age was associated with a decrease of the iodine number of the same wax. This relationship is in agreement with MARKLEY (**10**) that the melting point of a lipid substance may be raised by a decrease in unsaturation. The decrease in unsaturation of the waxes with age may have been brought about by oxidation of the wax compounds upon prolonged exposure to the atmosphere.

In the case of both *Sorghum halepense* and *Phoenix dactylifera,* the iodine number of the non-waxes increased through several stages of the early growth. However, in the most mature leaves the degree of unsatura-

TABLE I

EXTRACT YIELDS, CUTICLE THICKNESS, AND CHARACTERISTICS OF THE WAX AND NON-WAX FRACTIONS OF ELEVEN SPECIES BY AGE OF THE PLANT

SPECIES	AGE	CUTICLE THICKNESS	WAX					NON-WAX		
			YIELD	M.P.	IODINE no.	ACID no.	ESTER no.	YIELD	ACID no.	ESTER no.
		μ	%	°C				%		
Phragmites	young	2.6	0.16	80.0	1.17	9.41	52.5	0.20	45.23	150.5
communis	old	1.75	0.05	80.0	0.79	26.00	57.2	0.22	29.35	218.0
Acacia	young	2.27	0.10	77.0	5.19	11.29	53.3	0.15	22.19	0.0
greggii	old	2.2	0.32	79.5	0.39	6.05	75.0	0.14	27.83	111.7
Acacia	young	.....	0.15	77.0	2.65	5.04	69.6	0.16	70.58	65.6
vernicosa	old	.....	0.30	75.0	2.26	15.25	73.4	0.15	72.36	172.4
Acacia	young	2.27	0.41	71.0	5.65	4.11	81.5	0.29	22.45	109.3
constricta	old	2.27	0.30	75.0	1.11	9.06	79.2	0.20	18.82	3.6
Olneya	young	2.3	0.30	74.0	1.26	2.08	24.4	0.20	15.95	54.2
tesota	old	3.05	0.38	73.0	1.16	6.91	46.5	0.23	12.93	143.6
Asclepias	young	1.75	1.26	67.0	73.06	2.43	10.4	1.79	9.25	54.2
linaria	old	1.5	0.30	70.0	13.12	11.25	13.5	0.60	8.09	37.3
Beloperone	young	trace	0.37	70.0	13.02	5.35	16.6	0.22	19.22	66.2
californica	old	0.0	0.07	71.0	8.62	14.77	9.1	0.07	16.39	171.8
Baccharis	young	5.25	0.24	83.5	25.02	60.24	61.9	0.44	47.73	115.5
sarothroides ♀	old	4.5	0.15	80.0	17.04	52.70	36.6	0.42	38.89	93.0
Baccharis	young	3.5	0.06	78.3*	39.02	15.36	33.3	0.47	67.70	0.0
sarothroides ♂	old	5.25	0.21	78.5	53.85	14.25	52.4	0.57	33.81	4.5
Aplopappus	young	3.5	0.11	72.0	35.41	4.93	20.1	0.54	70.31	0.0
tenuisectus	old	4.8	0.34	72.0	23.97	12.07	15.2	0.56	59.96	0.0
Aplopappus	young	1.75	0.06	66.5	29.61	14.92	0.0	0.48	60.83	24.1
laricifolius	old	1.75	0.15	66.5	9.68	34.61	0.0	0.28	73.66	46.9

* Determined by capillary rod method

tion rapidly decreased in *S. halepense* and increased rapidly in *P. dactylifera*. The increase with age of unsaturation of the fatty constituents is in agreement with previous work (**8, 13**), but the significance of the rapid decrease of the iodine number with age of *S. halepense* is not known. It may be that as the old plant parts become desiccated, the unsaturated lipid compounds of this species are oxidized more rapidly by atmospheric oxygen.

The results of acid number determinations show that the amount of free acids in the wax fraction of some species decreased rapidly in the very young plant growth, and then increased during later growth. The acid content from the older material did not reach the amount found in the wax from the youngest leaves (figs. 1 and 2). These results may be correlated

TABLE II

EXTRACT YIELDS AND THICKNESS OF THE CUTICLE BY AGE OF *Sorghum halepense* AND *Phoenix dactylifera*

SPECIES	AGE	CUTICLE THICKNESS	YIELD	
			WAX	NON-WAX
		μ	%	%
	tops	.....	trace	0.33
	5 days	3.50	0.04	0.55
	10 days	3.50	0.05	0.58
Sorghum	15 days	3.67	0.06	0.56
halepense	20 days	3.60	0.05	0.34
	25 days	.....	0.05	0.31
	30 days	.....	0.05	0.25
	35 days	.....	0.04	0.19
	0 months	1.75	0.17	0.19
	3 months	2.60	0.15	0.16
Phoenix	12 months	3.50	0.10	0.15
dactylifera	18 months	3.50	0.19	0.13
	24 months	3.00	0.15	0.15
	36 months	3.00	0.15	0.24

with those of table I. The young plant material of *Acacia greggii*, and pistillate and staminate plants of *Baccharis sarothroides* were collected in a very young condition. The wax acid number from the youngest material was higher than that from the old material. Therefore the over-all trend appears as a decrease of acid number with age. This is in agreement with the over-all trend of the acid number of the wax fractions of *Sorghum halepense* and *Phoenix dactylifera*. The young plant material of the other species of table I was not collected in a very young stage of growth, so that the over-all trend in acid number with age of these species was an increase. This increase corresponds to the latter part of the acid number curves of *S. halepense* and *P. dactylifera*. Thus it may be said that without exception, in the species studied, the wax from very young leaves had a high acid content; that there was a rapid decrease during immediate growth there-

after; and that only during later periods of growth did the acid content of the wax increase. The significance of the rapid drop of wax acids in young leaves is not clear, but it may be associated with the synthesis of wax esters, as shown in figures 1 and 2.

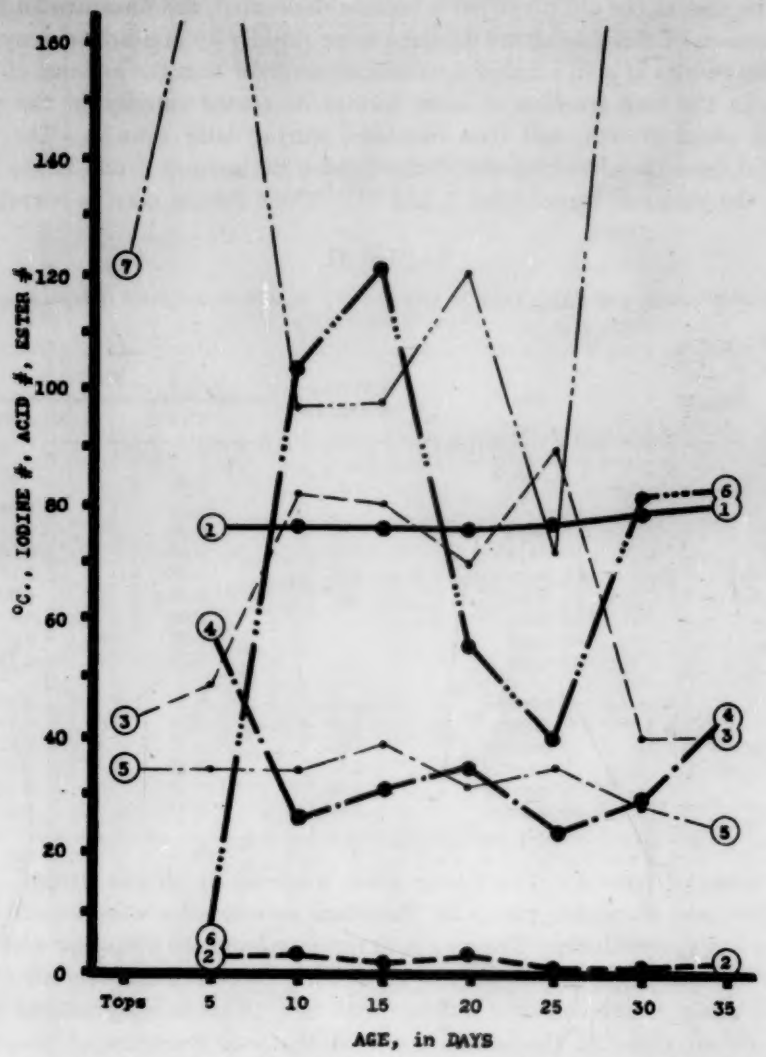

FIG. 1. Variations with age of *Sorghum halepense* of the characteristics of the wax and non-wax fractions (Wax: 1-1 melting point, 2-2 iodine no., 4-4 acid no., 6-6 ester no.; Non-wax: 3-3 iodine no., 5-5 acid no., 7-7 ester no.).

The free acids of the non-wax fraction appear to be related physiologically to the non-wax esters and the wax esters and acids. As has been discussed, the wax acid content increased with age after the very early stages of growth. The ester content of both wax and non-wax fractions also

increased with age of the plant. The non-wax acid content, on the other hand, remained about the same or decreased with age of the plant. These results suggest that cellular fatty acids (the non-wax acids) possibly are precursors of the esters of both fractions and the acids of the wax fraction. This would account for the decrease of the non-wax acid number and the development of the characteristics of the other constituents with age. The

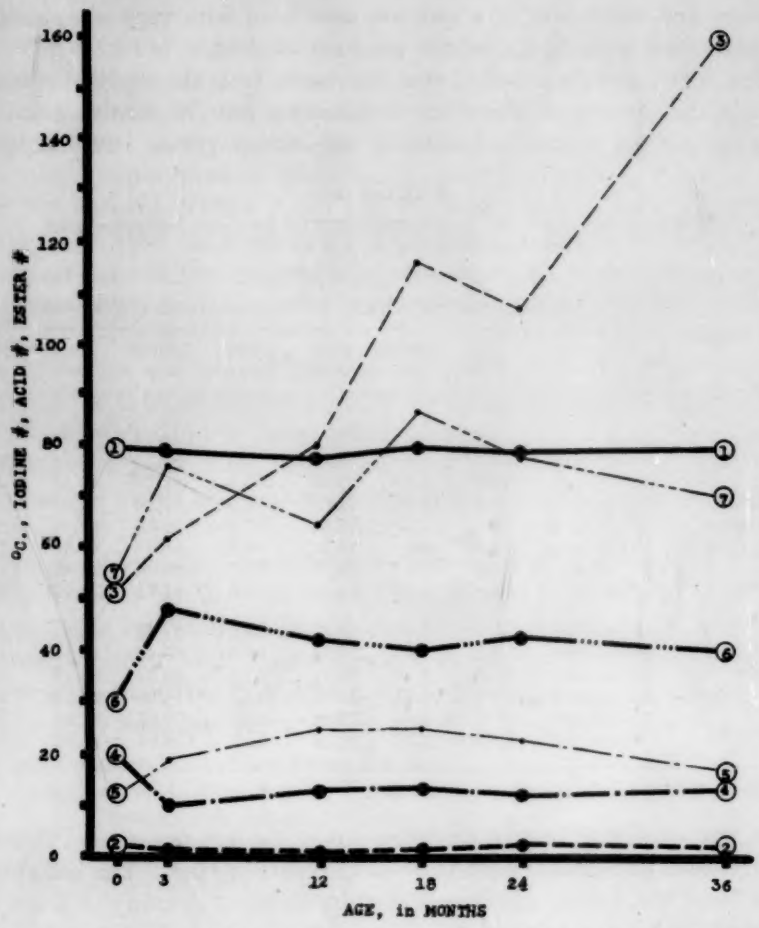

FIG. 2. Variations with age of *Phoenix dactylifera* of the characteristics of the wax and non-wax fractions (Wax: 1-1 melting point, 2-2 iodine no., 4-4 acid no., 6-6 ester no.; Non-wax: 3-3 iodine no., 5-5 acid no., 7-7 ester no.).

chemical reactions involved might be that cellular fatty acids condense to form higher molecular weight fatty acids that appear in the wax fraction. Reduction of the wax acids would form alcohols, and esterification would then form wax esters. Similar reactions could account for the increase of non-wax esters. The anabolism of the cellular fatty acids themselves, how-

ever, is a matter of conjecture, inasmuch as the only mechanisms which have been proposed have been largely theoretical.

Variations with age of the color and odor of the wax and non-wax fractions are readily interpreted. In general, the color and odor of the extracts corresponded to the pigmentation and odoriferous nature of the plant studied. No attempt will be made, however, to explain certain variations with age of hardness and brittleness of the wax fraction. Undoubtedly hardness and brittleness of a wax are associated with very complex inter- and intra-molecular factors which were not studied.

It will be noted from table I that the results from the study of staminate and pistillate plants of *Baccharis sarothroides* are frequently at variance with the general trends exhibited by the other species. The additional

TABLE III

THE WAX AND NON-WAX CHARACTERISTICS OF *Sorghum halepense* AND *Phoenix dactylifera* BY AGE OF THE PLANT

Species	Age of leaves	Wax				Non-wax		
		M.P.	Iodine no.	Acid no.	Ester no.	Iodine no.	Acid no.	Ester no.
		°C						
Sorghum halepense	tops	.....	.....	.....	.....	42.96	34.21	121.30
	5 days	76.5*	3.64	58.79	4.5	49.70	34.70	190.83
	10 days	76.5*	3.99	26.63	105.2	81.16	34.16	97.33
	15 days	76.3*	1.66	31.89	123.9	80.53	38.37	99.02
	20 days	76.3*	3.73	35.24	57.0	70.58	32.27	120.37
	25 days	76.8*	0.00	23.32	40.3	90.84	35.62	71.31
	30 days	78.5*	0.00	29.88	82.2	39.96	28.45	245.59
	35 days	79.8*	1.55	41.21	83.5	41.74	24.01	197.26
Phoenix dactylifera	0 months	80.0	2.19	20.17	30.0	51.12	12.52	54.12
	3 months	79.5	1.67	10.73	49.0	63.40	19.18	76.26
	12 months	77.5	0.97	13.96	43.0	80.54	25.17	65.15
	18 months	80.0	1.44	13.28	41.1	116.88	25.18	87.11
	24 months	78.5	2.44	12.41	43.0	107.01	22.93	78.60
	36 months	79.5	2.37	13.25	39.7	158.94	17.90	70.06

* Determined by the capillary rod method.

factor, sex, evidently showed precedence over the age factor, and therefore caused results at variance with those of the other species. The influence of sex on lipid metabolism apparently is quite strong. A study of the sexual relationships is being continued.

Summary

1. Thirteen species were studied as to the relationships of wax to plant age. After extraction with petroleum ether, the lipid extract was separated into wax and non-wax fractions. The yields and physical and chemical characteristics of each fraction were determined. All ages of plant material studied were also subjected to histological studies of the cuticle.

2. The yields of the wax and non-wax fractions by age varied differently with each species. The yield of the wax fraction varied directly with the thickness of the cuticle.

3. The wax melting point did not change markedly with age, but in some species there was a slight increase. This increase in melting point was correlated with a decrease in wax unsaturation. It was suggested that the decrease in unsaturation with age is caused by oxidation by atmospheric oxygen.

4. The degree of unsaturation of the non-wax fraction usually increased with age.

5. The amount of wax acids was found to decrease rapidly in young plants and then slowly increase as the plant matured. This variation of acid content appeared to be related to wax ester synthesis.

6. The amount of wax esters and acids and non-wax esters increased with age. It was considered that this might be correlated with the decrease or maintenance of the non-wax acid concentration as the plant matured. The cellular fatty acids appeared to be precursors of esters and wax fraction acids.

7. The color and odor of the wax and non-wax fractions were directly related to the type of pigmentation and odor of the plant.

8. No relationship was found between carbohydrate and lipid content.

9. The divergence from the general trends of the results of the staminate and pistillate plants of *Baccharis sarothroides* was believed to be associated with a sexual factor.

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ACCUMULATION OF FREE AMINO ACIDS AS A CHEMICAL BASIS FOR MORPHOLOGICAL SYMPTOMS IN TOBACCO MANIFESTING FRENCHING AND MINERAL DEFICIENCY SYMPTOMS[1]

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Introduction

The chemical mechanism whereby plants respond to modifications in environment resulting in alterations in morphology has on the whole remained obscure. However, whatever the cause, it is clear that each change in environment may result in the appearance of a whole aggregate of symptoms characteristically specific for each condition. No change in environment can be said to cause only a single symptom. Though individual symptoms may to some extent resemble each other with various environmental variations, the aggregate of symptoms is usually characteristic of each particular environment and plant in question.

The work discussed herein consists of studies on the chemical mechanism of symptom production in frenching and mineral deficiency of tobacco. The well defined aggregate of symptoms characteristic of these particular environments afford very favorable material for a study of the chemical mechanisms underlying morphological change.

The condition of tobacco known as frenching is a well known deformity. Growth of the terminal bud of the plant slows or stops and the slowly expanding new leaves develop a network type of chlorosis. In an extreme form the newly developed leaves become strap shaped because of the failure of the lamina to expand. Growth of axillary buds then starts but results only in the formation of similarly strapped leaves. Stem elongation whether of the main axis or of axillary branches is greatly inhibited in an extreme case.

STEINBERG (**12**) found that diffusates from numerous bacterial strains ordinarily present in soil are capable of causing changes in morphology of tobacco seedlings grown in aseptic culture. These alterations were in some cases suggestive of, but were not identical with, frenching in the field. Other experiments with amino acids in aseptic culture (**11**) (**13**) revealed that slightly excessive quantities of these and other natural metabolic compounds each caused the production of specific symptom complexes in tobacco seedlings. The conclusion naturally drawn was that release of excessive quantities of free amino acids into the tissues was a probable causative chemical factor in the production of morphological symptoms in the plant.

[1] Cooperative investigation by the Division of Tobacco, Medicinal and Special Crops, Bureau of Plant Industry, Soils, and Agricultural Engineering, Agricultural Research Administration, U. S. Department of Agriculture and the Maryland Agricultural Experiment Station.

Though none of the plants obtained with metabolites were identical with those showing frenching or mineral deficiency, they were, with respect to individual symptoms, often quite suggestive in appearance. Isoleucine particularly caused as close an approximation to frenching in the field as was to be expected in very young seedlings under the environmental conditions of flask culture in low intensity artificial light.

The suggestion (**11**) was therefore made that the mechanism of symptom production in mineral deficiencies might also depend on the same chemical mechanism as in frenching: accumulation of free amino acids. McMURTREY (**4**) has described the symptom-aggregates characteristic of mineral deficiencies in tobacco. However, while there are a great deal of analytical data on tobacco available, none are in a form suitable for deciding the accuracy of this assumption. Values for the individual free amino acids present in the normal and abnormal plants would be desirable.

It was considered probable, moreover, that if toxic concentrations of free amino acids are responsible in part or whole for the morphological alterations that appear, these would be reflected by a rise in total free amino acids in the plant. Since some of the amino acids are effective at dilutions far beyond the range of the Van Slyke method for determining a-amino nitrogen the accuracy of this assumption could only be determined by trial. There were some data available to bear out this belief, however, in the report of NIGHTINGALE, SCHERMERHORN, and ROBBINS (**9**) that sulphur deficiency in tomato caused an increase in amino acid nitrogen of the plant. RICHARDS and TEMPLEMAN (**10**) also found barley plants showing symptoms of phosphorus deficiency contained a "pronounced accumulation of amino nitrogen" as did those showing symptoms of potassium deficiency. On the other hand NIGHTINGALE, SCHERMERHORN, and ROBBINS (**8**) did not note any increase in amino nitrogen with potassium deficiency, nor did NIGHTINGALE, ADDOMS, ROBBINS, and SCHERMERHORN with calcium deficiency (**7**).

The work herein reported therefore consists of analytical determinations of free a-amino nitrogen in field plants showing symptoms of frenching, and mineral deficiencies of the following elements: K, Mg, Ca, P, B, and N. Growth experiments had already demonstrated the ability of externally applied natural metabolites to cause the appearance of manifold morphological changes. However this did not furnish definite proof of an excess of symptom-producing compounds in the plant tissues when the growth differences occurred.

Experimental methods

The tobacco plants used in the study of the effects of mineral deficiency on free amino acid content were grown at the Tobacco Experiment Farm at Upper Marlboro, Maryland. McMURTREY (**5**) has already described the experimental techniques, and the extent of the deficiencies attained. Samples from plants infected with tobacco mosaic were obtained from this locality also through the courtesy of Dr. E. E. Clayton. Samples of frenching soil

and plants used in this work were obtained from the George Merrick and Deshield farms in Prince Georges County, Maryland.

Individual leaves (25–100) from as many plants as conditions would allow were selected in sampling. The midribs were removed and the lamina quickly dried in a forced draft cabinet at 60–65° C. In the selection of samples both the position of the leaf on the plant and the age of the plant were taken into consideration, the age being based on time of flowering. In preparation for analysis the oven dried material was ground to pass a 40-mesh sieve and preserved in tightly stoppered containers. The method outlined by GARNER, BACON, BOWLING, and BROWN (**2**) was used for the determination of amino acid nitrogen. A correction for the content of moisture and soil material in the prepared sample was applied to the analytical data.

In barest essentials the analytical method consisted of the coagulation and removal of protein nitrogen with dilute acetic acid and heat, removal of amide and ammonia nitrogen by hydrolysis with 1.08 N-H_2SO_4 followed by distillation with 10% NaOH and determination of a-amino nitrogen by the Van Slyke nitrite method. The value for a-amino nitrogen in the residual solution so obtained corresponds to the number of amino acids present except that lysine gives a two-fold value, and proline and hydroxyproline fail to react.

Total a-amino nitrogen was determined in a similar manner except that 6.0 N-H_2SO_4 was used for hydrolysis instead of 1.08 N-H_2SO_4 in order to hydrolyze peptide, peptone, and proteose (**17**). Total a-amino nitrogen might also be called non-protein, a-amino nitrogen. Presumably it is an approximate measure of the sum total of units used in the synthesis and break-down of proteins present at the time of sampling.

FREE AMINO ACIDS IN FIELD PLANTS SHOWING SYMPTOMS OF FRENCHING OR MINERAL DEFICIENCY

The analytical data for field plants has been arranged according to the crop year. The samples dated as 1936, 1940, and 1945 (table I) were obtained for the study of other factors than those here under consideration (**1**), and prior to the time this study was begun. It will be noted that of the samples collected in 1936 and 1940, the plants were grown with a wide range in quantity of a potash fertilizer application. Definite symptoms of potash deficiency were noted where no potash was applied. Symptoms in 1940 were apparently more severe than in 1936 but seem to have been confined to the leaf tips and adjacent margins. Total free amino acid nitrogen did not vary from the controls in either year according to these data—a result which will be discussed in connection with the figures for later analyses.

The other values afford a comparison of normal and slightly frenched plants grown in 1945. It is seen that the leaves of frenched plants contained approximately double the free a-amino acid content of that in normal plants. Amide and ammonia nitrogen varied in a similar manner. Through the

courtesy of Dr. J. L. Stokes of Merck & Co., Rahway, N. J., it was possible to have the natural isomer of isoleucine estimated with *Streptococcus faecalis* (15). The isoleucine content, it can be computed, increased in the frenched plants by 50.0% in one case and by 49.6% in the other. It has been estimated that this is equivalent to an increase in the isoleucine content of 100 to 150 p.p.m., or about the same concentration of natural isoleucine (100 p.p.m.) in aseptic agar culture required to cause a similar morphological alteration artificially. The interpretation of the analogous increase in amide and ammonia of frenched plants requires additional data.

TABLE I

THE FREE A-AMINO NITROGEN CONTENT, IN DRY LEAF LAMINA OF MARYLAND MEDIUM BROADLEAF TOBACCO GROWN AT UPPER MARLBORO, MARYLAND, AS AFFECTED BY FRENCHING AND MINERAL DEFICIENCIES AT TIME OF FLOWERING*

Crop year	Treatment	Appearance of plants	Nitrogen constituents in moisture-free leaf lamina			
			Free a-amino nitrogen		Amide & ammonia nitrogen	Free l(+) – iso leucine
			Content	Relative content		
			%	%	%	%
1936	No potash added	Chlorotic leaf tips	0.108	125.6		
	Potash at 24 lbs. per acre	Slight leaf cupping	0.110	127.9		
	Potash at 120 lbs. per acre	Normal	0.086	100.0		
1940	No potash added	Chlorotic tips, dried margins	0.094	94.0		
	Potash at 30 lbs. per acre	Leaf tip chlorosis	0.093	93.0		
	Potash at 240 lbs. per acre	Normal	0.100	100.0		
1945	No potash added	Normal	0.098	100.0	0.053	0.034
	No potash added	Frenched, few strapped leaves	0.188	191.8	0.112	0.051
1945	No fertilizer added	Normal	0.095	100.0	0.057	0.034
	No fertilizer added	Frenched, few strapped leaves	0.210	221.1	0.115	0.050

* Samples were taken from plants at same stage of maturity when possible; the third and fourth leaves from top being selected. The greater part of the frenched material consisted of leaves showing reticular chlorosis and little or no strapping. Both normal and frenched plants were in the same plots: In the "no potash" plot slightly less than half the plants showed symptoms of frenching, and in the "no fertilizer" plot about 65% of the plants.

The material obtained in 1946 (table II) falls under four headings. Frenching again caused a significant rise in free a-amino nitrogen amounting to 53.1%. The frenched and normal plants were frequently growing side by side. The severely strapped leaves could not be used because of

absence of leaf lamina. Mosaic disease, however, seemed to have little influence on the content of free a-amino nitrogen; though other strains of virus characterized by leaf strapping may give a different response. The controls were separated from the diseased plants by a 36-foot fallow plot.

The results with mineral deficiencies seemed definite and conclusive. A deficiency in potassium, magnesium, calcium, phosphorus, nitrogen, or boron brought about marked accumulations of free a-amino nitrogen in each instance. Potassium deficiency caused almost a 7-fold increase, magnesium almost a 4-fold increase and calcium a doubling in free a-amino nitrogen. In each of these cases the plants and the leaves analyzed showed extreme symptoms of deficiency. The moderate symptoms shown with phosphorus,

TABLE II

THE FREE A-AMINO NITROGEN CONTENT IN DRY LEAF LAMINA OF MARYLAND MEDIUM BROADLEAF TOBACCO GROWN DURING 1946 AT UPPER MARLBORO, MARYLAND, AS AFFECTED BY FRENCHING AND MINERAL DEFICIENCIES

LOCATION OF PLOT OR DESIGNATION	TREATMENT	SYMPTOMS OF ABNORMALITY (SAMPLES TAKEN AUG. 13–22, 1946)	FREE a-AMINO NITROGEN	
			CONTENT	RELATIVE CONTENT
			%	%
Geo. Merricks Farm	Standard*	None	0.209	100.0
"	"	Extreme frenching	0.320	153.1
Rotation Series	Standard	None	0.122	100.0
"	"	Mosaic distinctly visible	0.127	104.1
Pure chemicals	Standard	None	0.098	100.0
	No B	Moderate boron deficiency	0.124	126.5
Fertilizer	Standard	None, but slight nitrogen deficiency	0.127	100.0
	No Ca	Extreme calcium deficiency	0.279	219.7
	No Mg	Extreme magnesium deficiency	0.486	382.6
	No N	Extreme nitrogen deficiency	0.167	131.5
	No P	Moderate phosphorus deficiency	0.188	148.0
	No K†	Extreme potassium deficiency	0.873	687.4

* These samples were prepared on August 13. Samples of dry soil collected on September 18 contained only a trace of amino nitrogen, whether the plant did or did not show symptoms of frenching.

† Total a-amino nitrogen was 0.949%.

and boron deficiency were accompanied with lesser increases in free a-amino nitrogen. It might be anticipated that a decrease in nitrogen supply would have the effect of minimizing any increase in free a-amino nitrogen, if it be assumed that accumulation is due to a block in protein breakdown as well.

Analyses of the material collected from the plants grown in 1947 (table III) gave results essentially identical with those of 1946. Marked increases in free a-amino nitrogen were found in each instance where a mineral deficiency caused the appearance of severe symptoms (magnesium, potassium). Moderate severity of deficiency symptoms were accompanied by a lesser accumulation of a-amino nitrogen (calcium, phosphorus). An insufficiency of nitrogen caused small decreases in a-amino nitrogen. Boron and sulphur

TABLE III

THE NON-PROTEIN FREE A-AMINO NITROGEN CONTENT IN DRY LEAF LAMINA OF MARYLAND MEDIUM BROADLEAF TOBACCO GROWN DURING 1947 AT UPPER MARLBORO, MARYLAND, AS AFFECTED BY FRENCHING AND MINERAL DEFICIENCIES

LOCATION OF PLOT OR DESIGNATION	TREATMENT	SYMPTOMS OF ABNORMALITY IN PLANTS AND ANALYTICAL SAMPLE	NITROGEN CONSTITUENTS IN MOISTURE-FREE LEAF LAMINA			
			FREE A-AMINO NITROGEN		TOTAL NON-PROTEIN A-AMINO NITROGEN	NON-PROTEIN COMBINED A-AMINO NITROGEN
			CONTENT	RELATIVE CONTENT		
			%	%	%	%
Fertilizer*	Standard	None	0.168	100.0	0.193	0.025
	No Ca	Moderate calcium deficiency in 75% of leaves sampled	0.311	185.1	0.356	0.045
	No Mg	Severe magnesium deficiency	0.561	333.9	0.617	0.056
	No K	Severe potassium deficiency	0.548	326.2	0.671	0.123
	No N	About 90% of leaves selected showed definite nitrogen deficiency	0.130	77.4	0.151	0.021
	No P	Small narrowed dark green leaves on small plants	0.244	145.2	0.298	0.054
	No B	Only 10% of leaves selected exhibited boron deficiency	0.163	97.0	0.188	0.025
	No Cl	None	0.211	125.6	0.275	0.064
	No S	Previous slight indication of sulphur deficiency	0.153	91.1	0.185	0.032
Merrick Farm*	Standard	None	0.432	100.0	0.492	0.060
	"	Moderate frenching	0.839	194.2	0.943	0.104
Deshield Farm†	Standard	Roots of normal plants	0.118	100.0	0.115	0
	"	Roots of severely frenched plants	0.130	110.2	0.158	0.028

* Middle leaves of plants were used for samples in the deficiency series and top leaves of the frenching plants from the Merrick farm.
† The roots collected at the Deshield farm for normal and frenched material were from plants showing frenched and normal suckers, respectively.

deficiencies were too slight to give any indication of an increase in amino acids. The influence of omission of chloride from the fertilizer mixture on a-amino nitrogen is of interest in view of its known effects (3) on drought resistance of tobacco and of leaf quality. Chlorine is not, so far as is known, an essential element in the nutrition of tobacco.

The quantities of a-amino nitrogen in plants showing symptoms of frenching and mineral deficiencies vary not only with the extent of the visible abnormalities in growth, but also with environmental conditions. Comparison of the percentages of free a-amino nitrogen in the controls of all the tables will reveal a variation between 0.053 and 0.432% with location and crop year.

The values for total non-protein, a-amino nitrogen parallel those for free amino nitrogen. They permit the computation by difference of non-protein combined a-amino nitrogen, which is relatively much smaller than the free a-amino nitrogen found. These values are presumed to include all non-protein a-amino nitrogen present in peptide, proteose, peptone, proline, and hydroxyproline. The nitrogen of the a-amino group of lysine, however, appears in the free a-amino nitrogen value.

The free a-amino nitrogen was approximately 76.7 to 100.0% of total non-protein a-amino nitrogen. The smallest value was given by plants where chloride was omitted from the fertilizer, the highest value with roots from normal plants. The other values can be readily computed. Their significance is not certain, since the variations were relatively small.

The last two rows deal with data obtained on the roots of frenched and normal plants. Only a slight difference in a-amino nitrogen (10.2%) was found. Greater weight should probably be assigned this difference than its magnitude would seem to demand, inasmuch as the normal plant was in process of forming frenched suckers and the frenched plant of becoming normal (suckers). It seems reasonable to assume that a comparison between better defined samples would show a definitely greater difference in free a-amino nitrogen.

Discussion

Further verification of the postulated free amino acid mechanism of symptom production in the tobacco plant is afforded to some extent by the analytical data on plants suffering from mineral deficiencies. In every case except perhaps boron, and nitrogen, the symptoms of mineral deficiency were paralleled by large abnormal increases in the free amino acids of the leaf tissues.

These exceptions may of course be due to the toxic accumulations of metabolites not containing a-amino nitrogen (13). Other explanations also exist however. One such is that the symptoms of boron deficiency were too mild and that too much normal tissue was included in the analytical samples. Plants with moderate minus boron symptoms (table II) gave an increase of 26.5% in free amino acid nitrogen even without rejection of more

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normal tissue from the analytical sample. A similar situation was encountered with minus potassium plants. Negative results were first obtained similarly with minus potassium plants since the analytical samples consisted of better than 90% normal leaf tissue.

An important contribution by WADLEIGH and SHIVE (**16**) presents evidence however that the cells of minus boron cotton plants are the seat of protein degenerations and increased acidities. An accumulation of sugar and ammonia was interpreted by them as due to a block in amino acid syntheses by the plants. This response would therefore seem to indicate too mild a boron deficiency as the most probable and important reason for the slight accumulation of excessive free amino acids with boron deficiency. The contrast in analytical results distinguishing calcium from boron deficiency actually obtained despite rather similar symptoms of deficiency appears striking. On the other hand boron deficiency symptoms originating through accumulation of the more toxic amino acids such as hydroxyproline (3 p.p.m.) would give no indication of any increase with the analytical methods employed.

The negative results with nitrogen deficiency are probably to be attributed to the prevention of amino acid synthesis. Symptoms of nitrogen deficiency may therefore possibly be due to loss of nitrogen from chlorophyll as well as accumulation of non-nitrogen metabolites in excess. No symptoms of sulphur deficiency were present in the plants at the time these were sampled for analysis.

Chlorosis accompanying a mineral deficiency however can no longer be casually assumed as indicative of an essential function in chlorophyll formation. The evidence would indicate rather that chloroses of this type are due to destruction of chlorophyll by excessive accumulations of amino acids and other metabolites. The characteristic pattern exhibited by the plant will, it is assumed, be found to be dependent on the particular metabolites that accumulate in excess, and the effects of anatomical structure in determining the regions in which toxic concentrations are permitted to become effective.

The marked fluctuations in a-amino nitrogen and protein nitrogen of plants subject to mineral deficiencies seem to be a general phenomenon. It would appear to indicate that the macronutrients and at least some of the micronutrients participate directly in the primary activity of the cell, namely the formation and breakdown of amino acids, proteins and intermediate products. Molybdenum also has recently fallen into this category since MULDER (**6**) found it to be as essential for nitrate reduction in green plants as for *Aspergillus niger* Van Tiegh.; and for denitrification as well as nitrogen fixation by soil bacteria. The points at which blocks are thus set up in the interlocking successive reactions of nitrogen metabolism of the cell are presumably as characteristic of the element in deficient supply as are the morphological symptoms. Thus RICHARDS and TEMPLEMAN (**10**) concluded

that accumulation of free amino acids was due to breakdown of proteins in potash deficiency; and to inhibition of protein synthesis in phosphorus deficiency of barley. Nitrogen deficiency however led to " . . . little indication of departure from the usual protein cycle . . . . " A more adequate review of protein and carbohydrate responses to mineral deficiencies will shortly (**14**) appear.

The significance of variations in free a-amino nitrogen with alterations in environmental conditions is further emphasized by the results of a study by WOOD and PETRIE (**18**) with *Phalaris tuberosa* Link. These investigators reported that the interrelations between protein and amino acids are unaffected by changes in content of carbohydrates: sucrose, glucose, and fructose. Proteins they suggest, ". . . are formed from the whole of the amino acids in the cell."

Summary

Frenching was accompanied by a marked increase in isoleucine and other free amino acids in the leaf lamina of field plants of Maryland Medium Broadleaf tobacco. The assumption that the rise in free amino acids in frenching was the primary cause or mechanism of symptom production in the plant was also supported by the analytical data on field plants showing mineral deficiencies. Sharp increases in free amino acids accompanied calcium, magnesium, potassium and phosphorus deficiency, but not in nitrogen deficiency. Boron and particularly sulphur deficiencies were too slight to give definite chemical differences in tissues. It is probable that calcium, magnesium, potassium, phosphorus and perhaps boron function in amino acid and protein metabolism of the plant. Chloroses due to mineral deficiencies, excepting possibly magnesium and nitrogen, are attributed primarily to the direct toxic action of excessive accumulations of metabolites.

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CHANGES IN CHEMICAL COMPOSITION OF THE LEAVES AND ROOTS OF RED KIDNEY BEAN PLANTS TREATED WITH 2, 4-DICHLOROPHENOXYACETIC ACID[1]

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Introduction

Investigators (**2, 6, 7, 11**) have shown that there is a depletion of carbohydrates and an accumulation of nitrogen in plants treated with 2, 4-dichlorophenoxyacetic acid (2, 4-D). SELL *et al.* (**10**) have observed similar trends in the abnormally proliferated stem tissue of red kidney bean plants treated with 2, 4-D. Recently, LUECKE *et al.* (**4**) have reported that there are also pronounced changes in the content of some members of the vitamin B complex and carotene in the leaf and stem tissue of red kidney bean plants treated with 2, 4-D. In view of these observations analyses were made to determine whether the leaf and root tissues of plants treated with 2, 4-D showed the marked differences in amino acids, ether extract, carbohydrates, ash and crude fiber content observed in the abnormally proliferated stem tissue of the treated plants (**10**). The results of these analyses are presented in this report.

Materials and methods

SAMPLES OF LEAF AND ROOT TISSUES

Seeds of the red kidney bean were selected for uniformity of size and planted in 4-inch pots in the greenhouse. Each pot contained two plants which were treated when the first trifoliate leaves were expanding. Two replications of 200 plants each were used as a source of both treated and non-treated (control) plant material. One drop (.05 ml.) of a 0.1% solution of 2, 4-D was applied to the base of the blade of a primary leaf of each treated plant (**6**). The plants were harvested six days after treatment. At this time the stem tissue had proliferated considerably but there were no signs of necrosis. The material was dried according to the procedure given by LINK (**3**) and then segregated into the various parts, the hypocotyl, first internode and leaf petioles being grouped together as stem tissue. Analyses were made of leaves and roots separately.

ANALYTICAL METHODS

Amino acids were determined microbiologically with the organisms *Lactobacillus arabinosus, Streptococcus faecalis* and *Leuconostic mesenteroides.* The media used in the various determinations were essentially the same as

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those described by SAUBERLICH and BAUMANN (**8**). Samples were prepared for assay by the method described by STOKES *et al.* (**12**), with the exception of the tryptophane samples which were prepared according to the method of WOOLEY and SEBRELL (**13**). Carbohydrates were determined by the procedure outlined by SELL *et al.* (**9**). The methods of analysis for nitrogen, crude fiber, ether extract, and ash were those given in A.O.A.C. (**1**).

Results and discussion

The results of the chemical analyses are summarized in tables I, II, and III. Each entry is a single determination on each replicate.

The data in table I, expressed as per cent. of amino acid or protein in the sample show the following trends: The leaf tissue of the treated plants contained slightly less protein and amino acids than that of the controls.

TABLE I

EFFECT OF 2,4-DICHLOROPHENOXYACETIC ACID ON THE AMINO ACID CONTENT OF THE LEAVES OF RED KIDNEY BEAN PLANTS
(EXPRESSED ON A DRY WEIGHT BASIS)

CONSTITUENT	TREATED		NON-TREATED		TREATED		NON-TREATED	
	IN THE SAMPLE		IN THE SAMPLE		IN CRUDE PROTEIN		IN CRUDE PROTEIN	
	REPLICATE 1	REPLICATE 2	REPLICATE 1	REPLICATE 2	REPLICATE 1	REPLICATE 2	REPLICATE 1	REPLICATE 2
	%	%	%	%	%	%	%	%
Protein (Nx6.25)	30.71	28.78	31.24	30.89				
Leucine	2.00	1.93	2.25	2.23	6.51	6.71	7.20	7.22
Isoleucine	1.46	1.45	1.63	1.59	4.75	5.00	5.21	5.15
Valine	1.64	1.61	1.78	1.78	5.34	5.59	5.69	5.76
Phenylalanine	1.33	1.28	1.47	1.43	4.33	4.45	4.71	4.63
Histidine	.70	.71	.84	.85	2.27	2.45	2.70	2.75
Arginine	1.54	1.54	1.76	1.73	5.01	5.35	5.63	5.60
Lysine	1.29	1.26	1.44	1.41	4.20	4.38	4.61	4.56
Tryptophane	.32	.36	.42	.43	1.04	1.25	1.34	1.38
Methionine	.24	.20	.29	.24	.78	.69	.93	.79
Threonine	1.25	1.24	1.35	1.41	4.07	4.31	4.32	4.56
Aspartic Acid	1.73	1.86	1.90	2.04	5.63	6.46	6.08	6.60

Expressing the data as per cent. of amino acid in crude protein a similar trend was observed for most of the amino acids. In table II it is noted that the roots of the plants treated with 2, 4-D contained about the same percentage of protein as did the controls. Slightly lower percentages of leucine, isoleucine, phenylalanine, lysine, tryptophane, methionine, threonine and aspartic acid in the sample and in the crude protein were observed in the root tissue of the treated plants. Valine, histidine and arginine were present in approximately the same percentage in both the treated plants and the controls.

The data in table III show a depletion of non-reducing sugars in the

TABLE II

EFFECT OF 2,4-DICHLOROPHENOXYACETIC ACID ON THE AMINO ACID CONTENT OF THE ROOTS OF RED KIDNEY BEAN PLANTS
(EXPRESSED ON A DRY WEIGHT BASIS)

CONSTITUENT	TREATED		NON-TREATED		TREATED		NON-TREATED	
	IN THE SAMPLE		IN THE SAMPLE		IN CRUDE PROTEIN		IN CRUDE PROTEIN	
	REPLICATE 1	REPLICATE 2	REPLICATE 1	REPLICATE 2	REPLICATE 1	REPLICATE 2	REPLICATE 1	REPLICATE 2
	%	%	%	%	%	%	%	%
Protein (Nx6.25)	22.90	21.80	22.84	22.62				
Leucine	1.01	1.02	1.11	1.15	4.40	4.67	5.22	5.51
Isoleucine	.87	.86	.96	1.02	3.78	3.95	4.51	4.88
Valine	.98	1.21	1.10	1.15	4.30	5.55	5.16	5.47
Phenylalanine	.60	.61	.64	.63	2.61	2.77	2.99	3.01
Histidine	.41	.44	.39	.40	1.81	2.00	1.83	1.90
Arginine	.71	.84	.77	.81	3.09	3.84	3.61	3.86
Lysine	.75	.79	.96	1.00	3.28	3.63	4.52	4.74
Tryptophane	.05	.09	.15	.12	.24	.43	.67	.58
Methionine	.11	.10	.14	.15	.50	.45	.64	.70
Threonine	.76	.76	.83	.86	3.30	3.49	3.88	4.07
Aspartic Acid	1.10	1.14	1.42	1.43	4.81	5.21	6.67	6.82

roots and leaves of the plants treated with 2,4-D. Other investigators have reported similar trends in other plants (**5, 7, 11**). No significant difference was noted in the percentage of reducing sugar in both the roots and leaves

TABLE III

EFFECT OF 2,4-DICHLOROPHENOXYACETIC ACID ON THE CARBOHYDRATE, CRUDE FIBER, ETHER EXTRACT AND ASH CONTENT OF THE LEAVES AND ROOTS OF RED KIDNEY BEAN PLANTS
(EXPRESSED ON A DRY WEIGHT BASIS)

CONSTITUENT	LEAVES				ROOTS			
	TREATED		NON-TREATED		TREATED		NON-TREATED	
	REPLICATE 1	REPLICATE 2	REPLICATE 1	REPLICATE 2	REPLICATE 1	REPLICATE 2	REPLICATE 1	REPLICATE 2
	%	%	%	%	%	%	%	%
Reducing Sugar	0.00	0.00	0.00	0.00	0.02	0.00	0.00	0.43
Non-reducing Sugar	0.00	0.25	0.82	1.14	0.00	0.00	1.53	2.98
Starch	1.77	1.76	1.87	1.61	2.13	2.01	2.02	2.44
Polysaccharide*	5.11	4.54	5.22	6.50	9.31	10.07	7.97	9.43
Crude Fiber	15.35	14.86	14.27	13.39	20.15	19.04	20.52	20.26
Ash	19.48	17.30	18.23	16.67	20.26	17.17	17.81	15.12
Ether Extract	2.59	2.77	3.43	2.80	1.32	1.52	1.33	1.59
Unsaponifiable Residue of the Ether Extract	1.59	1.40	1.28	1.60	1.06	1.08	1.07	.73
Fatty Acids by difference of the Ether Extract	1.00	1.37	1.15	1.20	.26	.44	.26	.86

* Acid hydrolyzable residue from the starch determination.

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of the treated and non-treated plants. The percentages of starch, polysaccharides, fatty acids, crude fiber, ash, ether extracts and unsaponifiable material were essentially the same in the leaves and roots of both the treated and non-treated plants with the exception of a slightly higher percentage of crude fiber in the leaf tissue of the treated plants.

The results of this investigation and previous work (**10**) show that the greatest differences in the quantities of proteins, amino acids, ether extract, carbohydrates, ash and crude fiber occur in proliferated abnormal stem tissue. The changes in the quantities of the constituents in the root and leaf tissue of the treated plants were not as pronounced as those in the stem tissue. However, slightly lower percentages of protein and amino acids were noted in the leaf tissue of the 2,4-D treated plants. A similar trend was observed in the roots for leucine, isoleucine, phenylalanine, lysine, tryptophane, methionine, threonine and aspartic acid. The tremendous increase of protein and amino acids (**10**) in the stem tissue of the plants treated with 2,4-D may be accounted for in part by the translocation of some of the amino acids from the roots and leaves.

Summary

1. The leaves of the red kidney bean plants treated with 2,4-D contained lower percentages of protein and of most of the amino acids in the sample and in the crude protein than did the controls. The percentages of protein, and the amino acids valine, histidine and arginine in the sample and in crude protein were almost the same in the roots of the treated and non-treated plants while the other amino acids were slightly less in the roots of the treated plants. The reduction of most of the amino acids in the root and leaf tissue of the treated plants may be due in part to the translocation of these substances to the stem tissue.

2. A depletion of non-reducing sugar was observed in both the leaves and roots of the treated plants. There was no significant change in the percentage of reducing sugar. The percentages of starch, polysaccharide, crude fiber, ash, ether extract, unsaponifiable material and fatty acids were practically the same for both the leaves and roots of the treated and non-treated plants.

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CHLOROPHYLL STUDIES ON BARLEY MUTANTS

HARRY R. HIGHKIN

(WITH FOUR FIGURES)

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Introduction

Those species of plants in which there is considerable information on the genetics of chlorophyll deficiency furnish favorable material for a study of physiological control by the gene over the production of chlorophyll and related pigments.

This study is a preliminary survey of the pigments of a number of genetically different chlorophyll mutant stocks in barley.

RABINOWITCH (**8**) has pointed out that apart from the isolated observation of INMAN and BLAKESLEE (**5**), who reported a chlorophyll with a different absorption spectrum from ordinary chlorophyll in an X-rayed mutant of Datura, the chlorophyll of all higher land plants investigated has been found to consist of the two components—the blue-green chlorophyll a and the yellow-green chlorophyll b.

However, W. H. EYSTER (**3**) in 1924 reported the "common" occurrence of four types of pigment-deficient plants in maize. These plants were those which: (1) contained carotin, xanthophyll and chlorophyll a but lacked chlorophyll b; (2) contained carotin, xanthophyll and chlorophyll b but lacked chlorophyll a; (3) contained carotin, xanthophyll and chlorophyll a and b in varying amounts; and (4) contained carotin and xanthophyll but lacked both chlorophyll a and chlorophyll b. Eyster reports that in his studies plants which lack either chlorophyll a or chlorophyll b are unable to synthesize sufficient carbohydrates to grow to maturity.

Except for this one report by Eyster, the only organisms in which a chlorophyll component has been found lacking are some of the algae, (**2**), (**6**), (**12**), (**13**), (**15**), (**16**), (**18**).

The descriptive term "chlorina" has been applied to certain pale green chlorophyll-deficient characters in barley. The color may vary from pale green to yellow green in different chlorinas. Several of those chlorophyll-deficient chlorinas have been described by ROBERTSON (**10**), NILSSON-EHLE (**7**) and others. Most of them, when studied in crosses with normal green plants, differ from the normal by one recessive gene. ROBERTSON (**9**) described one chlorina, however, which is apparently maternally inherited. When this chlorina, Coast V, is used as the female parent and crossed with green, the F_1, F_2 and F_3 are all chlorinas. The reciprocal cross, using it as the male parent, results in normal F_1, F_2 and F_3.

Materials and methods

The eight stocks of barley used were supplied by Dr. D. W. Robertson of the Colorado College of Agriculture and Mechanics. Three of these were segregating while the remaining five were homozygous for the chlorina character. All were viable and capable of growing to maturity. The following is a list of the stocks, together with the genetic symbols describing their other characters included for identification. (These other plant characters do not directly concern the pigments. A key to these symbols can be found in (**11**).) The symbols *f* and *fc* are used for the chlorina characters for which the linkages are known. The others have not been located.

Stock #1 Trebi IV segregating chlorina (OovvbbNNkkRRss)
Stock #2 28.3398 chlorina (vvbbNNkkSSblblrr)
Stock #3 Colsess V; chlorina fc in linkage group 7. (vvbbNNkkllBlBlRRoo)
Stock #4 Coast V; maternal seed (chlorina) maternally inherited
Stock #5 Minn 84-7 chlorina; f in linkage group 1. (vvbbNNkkiiRRSS)
Stock #6 Comfort II chlorina (vvbbNNkkrrSS)
Stock #7 II26-361-4(23) segregating chlorina
Stock #8 Coast Dwarf (2) segregating chlorina

These eight stocks were planted in February 1946 in a mixture of peat and sand in the greenhouse at about 27° C. Sixteen days after planting, the seedlings in each culture were harvested in three separate lots for chlorophyll extraction and dry weight determinations. This material was used for spectroscopic analysis.

Seeds of stock #2 chlorina, along with #1, #7 and #8 to furnish normals as controls, were planted again in April 1946 and harvested in triplicate 16 days later to furnish material for a chromatographic analysis of the pigments. Again in May 1946 the same stocks were grown to furnish material for separation of the pigments. This time, however, the plants were grown for 30 days, the procedure otherwise being the same as for the first two groups. Wherever segregation was found, the green and chlorina plants were harvested separately. The green plants segregating in stocks #1, #7 and #8 were considered as normal checks.

The ether extracts used in the spectroscopic analysis of chlorophyll were obtained by a slight modification of the method described by GRIFFITH and JEFFREY (**4**). Enough seedlings were harvested for one analysis at a time, brought to the laboratory from the greenhouse as rapidly as possible, and a weighed amount (eight to twelve grams) of the entire seedling (exclusive of the roots) placed in a Waring Blendor cup. A slight amount of calcium

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carbonate and 50 cc of acetone were added. During the process of maceration the sides of the cup were washed down to ensure complete extraction. After about ten minutes in the blendor the macerate was filtered through paper in a Buchner funnel and both cup and paper were washed with 25 cc portions of acetone, making a total volume of 125 cc of extract.

A 10 cc aliquot of the acetone extract and 10 cc of peroxide-free ether were put into the counter-current washing apparatus designed by GRIFFITH and JEFFREY (**4**) and washed with water. The final ether extract was made up to a volume of 25 cc.

To make a complete separation of the pigments from stock #2 the following method was used:

The plant material was macerated in a Waring Blendor with a few chips of ice, a few grams of $MgCO_3$ and cool acetone for three to five minutes. The mixture was filtered by suction in a Buchner and the filtrate put into a separatory funnel containing petroleum ether and a few chips of ice. The chlorophyll was washed in a counter-current washing apparatus (**4**) once with water and six times with 80% MeOH to remove the xanthophyll. It was then washed again with water and dried over anhydrous sodium sulphate in a refrigerator for 30 minutes.

The petroleum ether mixture was filtered in a Buchner prepared by packing a layer of powdered sugar on filter paper and a packed layer of Hyflo Super Cell on top of the sugar. The layered material was wet with petroleum ether and then the chlorophyll solution was filtered under suction and washed exhaustively with chilled petroleum ether to remove the carotin. When the filtrate was colorless the solid material with the adsorbed pigment was removed and the pigment eluted with ethyl ether and evaporated almost to dryness, then taken up with petroleum ether to make a solution containing 70% petroleum ether and 30% ethyl ether. This solution was used for chromatographic separation on a powdered sugar column, by a method to be described later.

The absorption spectra were measured on a Beckman spectrophotometer and the chlorophyll a and b content of the solution determined by use of the equation published by COMAR and ZSHEILE (**1**):

$$\text{Chlorophyll a (mg/l)} = 9.93 \log_{10} I_o/I_{660} - 0.777 \log_{10} I_o/I_{642.5}$$
$$\text{Chlorophyll b (mg/l)} = 17.6 \log_{10} I_o/I_{642.5} - 2.81 \log_{10} I_o/I_{660}$$

For comparisons, standard errors were calculated, using the following formulae (**17**):

$$\text{s.e.} = \sqrt{\frac{S(fx^2) - S(fx)\bar{x}}{N-1}}$$

$$\text{and } S.E._{diff.} = \sqrt{s.e._a^2 + s.e._b^2}$$

P values corresponding to the various values of diff./$S.E._{diff.}$ were determined from the tables in TRELOAR (**17**). A P value was considered to be significant if smaller than .05.

All chlorinas were compared with the average of the three lots of normals from the segregating strains. The results would have been similar if any one of the normal lots had been used.

Experimental results

The different strains of chlorinas varied in the degree of chlorosis shown at the 27° C temperature at which they were grown. Only those from stocks #6 and #8 were difficult to distinguish from the normals at that temperature. Stock #7 was an extremely pale chlorina.

Pigment analyses of three samples were run for each of the seven genetic stocks, the data being summarized in table I.

TABLE I

SUMMARY OF SPECTROPHOTOMETRIC DETERMINATIONS OF PIGMENT CONTENTS OF NORMAL CHLORINA STOCKS EXPRESSED AS MILLIGRAMS PER GRAM OF OVEN-DRY WEIGHT

STOCKS	CHLOROPHYLL A	CHLOROPHYLL B	TOTAL CHLOROPHYLL	% CHLOROPHYLL A	RATIO A/B
NORMALS					
1N	6.19 ± .184	2.44 ± .071	8.63 ± .339	71.7	2.54 ± .020
7N	5.91 ± .700	2.56 ± .368	8.44 ± 1.072	70.1	2.33 ± .052
8N	6.48 ± .329	2.09 ± .145	8.57 ± .464	75.6	3.12 ± .136
AVERAGE	6.19 ± .335	2.35 ± .256	8.55 ± .183	72.5	2.66 ± .425
CHLORINAS					
1	3.10 ± .071	0.90 ± .026	4.00 ± .071	77.5	3.44 ± .133
2	8.10 ± .725	0.34 ± .114	8.43 ± .840	96.1	23.83 ± .479
3	7.96 ± .530	3.00 ± .208	10.97 ± .771	72.6	2.65 ± .040
4	6.31 ± .157	2.50 ± .235	8.80 ± .387	71.6	2.59 ± .220
5	5.72 ± .191	1.68 ± .211	7.73 ± .366	74.0	3.01 ± .118
6	4.03 ± .329	1.33 ± .105	5.36 ± .435	75.2	3.17 ± .310
7	0.00	0.00	0.00	0.0	0.00
8	1.05 ± .022	0.41 ± .251	1.46 ± .141	71.6	2.55 ± .262

Examination of this table shows that in all cases except stock #2 the ratio of chlorophyll a to chlorophyll b fell within the ratio given by WILLSTÄTTER and STOLL (**19**), i.e., 2.93 ± 0.5–0.6. In addition, the extreme chlorina segregating in stock #7 was entirely lacking in both chlorophyll components. The chlorina was slightly yellower than albino and gave an absorption peak at 450 mu and no absorption between 600 and 700 mu.

Table II presents the statistical analysis of the differences between the chlorinas and the normals. In chlorophyll a content, the three normal lots were very similar. In chlorophyll b content, two of them (#7 and #8) were significantly different, but neither of them deviated significantly from the mean. The chlorinas from segregating lines (#1, #7 and #8) were compared with their normal sibs and with the average of the three normal lots. The remaining chlorinas were compared only with the average of the three normal lots.

The quantities of chlorophyll a and chlorophyll b in chlorina stocks #4

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TABLE II

SUMMARY OF COMPARISONS OF CHLOROPHYLL A AND B CONTENTS AND TOTAL CHLOROPHYLL IN NORMAL PLANTS AS COMPARED WITH CHLORINA PLANTS, TOGETHER WITH THE CORRESPONDING P VALUES AS MEASURES OF SIGNIFICANCE

P = .01 OR SMALLER INDICATES HIGHLY SIGNIFICANT DIFFERENCES

CHLORINA STOCKS	CHLOROPHYLL A			CHLOROPHYLL B			TOTAL CHLOROPHYLL		
	DIFFERENCE ± S.E.	DIFFERENCE / S.E.	P	DIFFERENCE ± S.E.	DIFFERENCE / S.E.	P	DIFFERENCE ± S.E.	DIFFERENCE / ± S.E.	P
1*	+3.09 ± .197	15.69	<.0001	+1.54 ± .076	20.26	<.0001	+4.63 ± .346	13.38	<.0001
1	+3.09 ± .342	9.04	<.0001	+1.45 ± .258	5.62	<.0001	+4.55 ± .196	23.21	<.0001
2	−1.91 ± .799	2.39	.0168	+2.01 ± .281	7.15	<.0001	+0.12 ± .860	0.14	.8887
3	−1.78 ± .627	2.84	.0045	−0.65 ± .330	19.6	<.0001	−2.42 ± .792	3.06	.0022
4	−0.12 ± .370	0.32	.7490	−0.15 ± .348	0.43	.6672	−0.25 ± .428	0.58	.5619
5	+0.47 ± .386	1.22	.2225	+0.34 ± .333	1.02	.3077	+0.82 ± .404	2.00	.0455
6	+2.16 ± .469	4.61	<.0001	+1.02 ± .105	9.71	<.0001	+3.19 ± .472	6.76	<.0001
8*	+5.43 ± .330	16.5	<.0001	+1.68 ± .290	5.79	<.0001	+7.11 ± .485	14.65	<.0001
8	+5.14 − .336	15.3	<.0001	+1.94 − .359	5.40	<.0001	+7.09 − .231	30.69	<.0001

* Compared with the values for the normal sibs. All others are compared with the average for the three normal stocks.

N.B. Chlorina #7 was omitted from this table since it contained no chlorophyll.

and #5 were not significantly different from those in normal stocks. Chlorophyll a and chlorophyll b contents in the chlorina stocks of #1, #6 and #8 were lower than in the normals, the differences being highly significant. Both chlorophyll components in stock #3 were higher than in the normal, the difference being significant for chlorophyll a ($P = .0045$) and highly significant for chlorophyll b ($P = < .0001$).

Chlorina #2 had a significantly higher amount of chlorophyll a than did the normal plants and a significantly lower amount of chlorophyll b. The percentage of chlorophyll a on a dry weight basis was 96% of the total chlorophyll and the mean ratio of a to b was 23.83, compared with 72.5% and a ratio of 2.66 for the average of the normal lots.

Since chlorina #2 was a selection from the cross Lion × Coast 1 (personal correspondence with D. W. Robertson), comparisons between both parents and the abnormal type were made. The material was treated as previously described, and the ether solution used for spectroscopic analysis. One of the parents, Coast 1, segregated albino in a three to one ratio (72 normal and 28 albino). The albino seedlings were harvested eleven days after planting since it seemed doubtful whether they would live for the full 16 days. The extract was yellow and had a peak at 450 mu and no absorption in the area between 600 and 700 mu.

Figure 1 shows the absorption curves of one of the normal parents and the chlorina #2 mutant.

Because of the interesting deviation of stock #2 from the expected ratio, a second lot together with normal plants of stocks #1, #7 and #8 as controls were grown under environmental conditions as similar to those of the first test as was possible, *i.e.*, the same type of soil, the same approximate temperature and the same conditions of humidity. The light period was different since the first analysis was made in February and the second in April with no supplementary light added at either time. The plants were grown for 16 days, harvested, extracted and a 10 cc sample from each washed and used for spectroscopic analysis as previously described. The remainder of the acetone extracts from the three samples of the chlorina stock were then combined, made to a total of 500 cc and gradually added to petroleum ether and washed with distilled water until freed of the acetone. The petroleum ether solution was dried over anhydrous sodium sulphate and concentrated to 50 cc volume. Twenty cc of this solution were used for a chromatographic analysis (**14**) of the pigments in the solution by adding it to a powdered sugar column 26 mm in diameter and 300 mm in length and forcing it through the column with air pressure.

The column contained only one blue-green band, with two narrow yellow bands above it and a number of yellow bands below it. The column was removed from the glass tubing and cut into three parts. Fraction I contained that portion of the top of the column which normally would contain the chlorophyll b band and here contained yellow bands (fig. 2) which to

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the author's knowledge have not been observed or reported and will (on separation and identification) be reported in a later communication. Investigations spectrally and with adsorption are in progress to learn whether these bands are present and obscured in normal plant chromatograms or

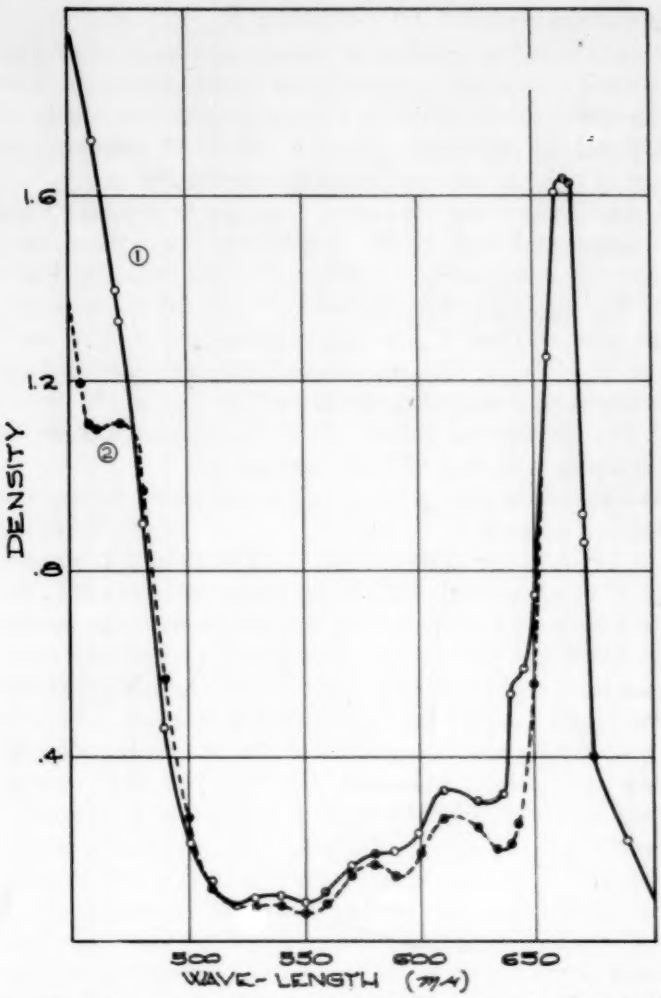

FIG. 1. Absorption curves of crude chlorophyll extracts in ether showing normal parent (1) and mutant chlorina #2 (2). Optical densities adjusted to same values at 660 mu.

whether they are a new pigment characteristic of the deficient mutant. Fraction II contained only the blue-green band; and fraction III everything below. The pigments were eluted from the sugar with ethyl ether and then made to volume.

Fraction I contained no green pigments and the absorption spectrum

showed a peak only at 440 mu. The curves of Fractions II and III are essentially the same and are both similar in the red region of the spectrum to the curve obtained for chlorophyll a in an ether solution (fig. 3) indicating the complete absence of chlorophyll b. Lack of similarity in the short range in curves of fractions II and III with the chlorophyll a curve was due to yellow pigment contamination. The normal control, stock #1, did contain chlorophyll b in Fraction I.

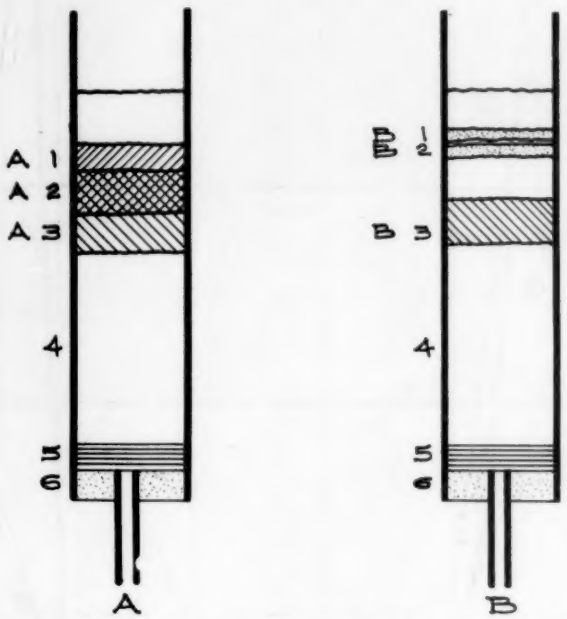

FIG. 2. Diagram showing adsorption columns of normal and chlorina stock #2. (A—normal; B—chlorina #2).

A1 Yellow-green chlorophyll b band.
A2 Mixture of both chlorophylls a and b.
A3 Blue-green chlorophyll a band.
B1 Unknown yellow band.
B2 Unknown yellow band.
B3 Blue-green chlorophyll a band.
4 Carotinoids.
5 Cotton.
6 Stopper.

For a final analysis of stock #2 a complete separation of the pigments was made following the method previously described. Only one green pigment appeared on the sugar column. The absorption curve obtained for it on the Beckman photospectometer (fig. 4) shows the pigment to be chlorophyll a.

Although the absorption curve is almost identical with that of a pure chlorophyll a solution as described by ZACHEILE and COMAR (**20**), the color

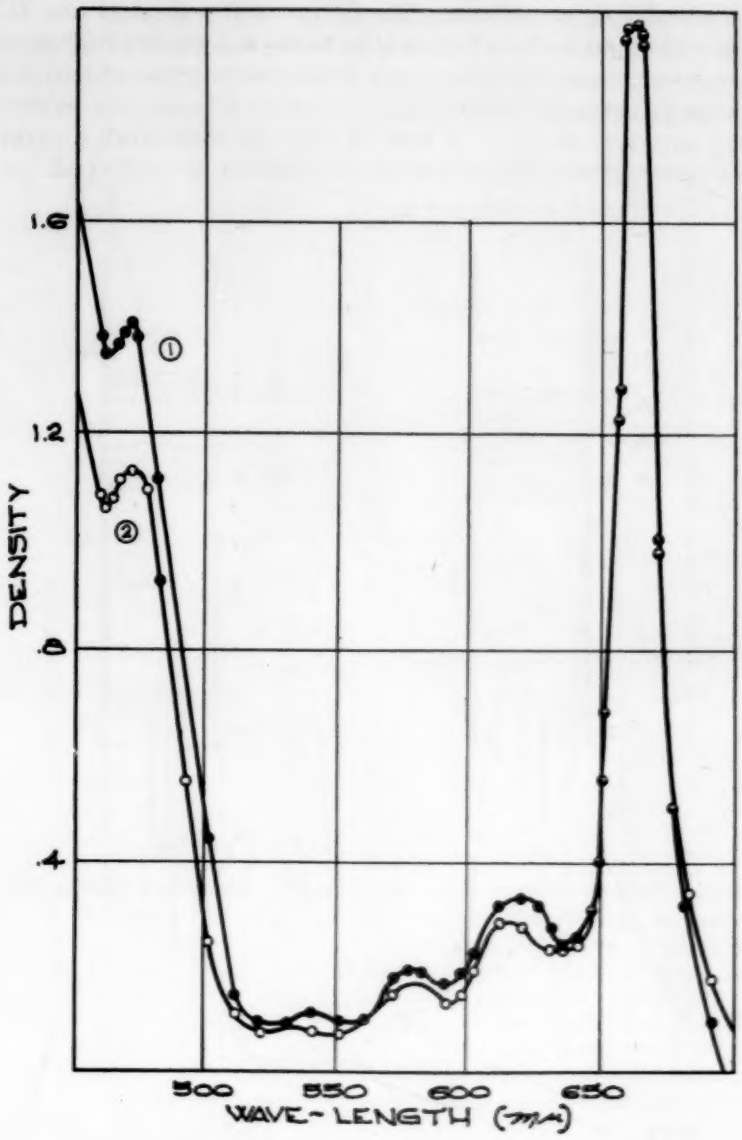

FIG. 3. Absorption curves of crude ether extracts showing fractions II (1) and III (2) taken from powdered sugar adsorption column.

is not quite the same as that of a pure chlorophyll a solution prepared from normal plant material. The solution was more yellow green than blue green. The phase test was positive although the transient yellow color was extremely rapid as compared with chlorophyll a from normal plants.

Discussion

The results were obtained under greenhouse light conditions and the relatively high temperature of 27° C. In the chlorotic segregant from stock #7, no chlorophyll was present. Analysis of chlorina plants in stocks #1, #6 and #8 indicates a total chlorophyll content very much lower than in normal plants, while chlorina #3 which is phenotypically similar to chlor-

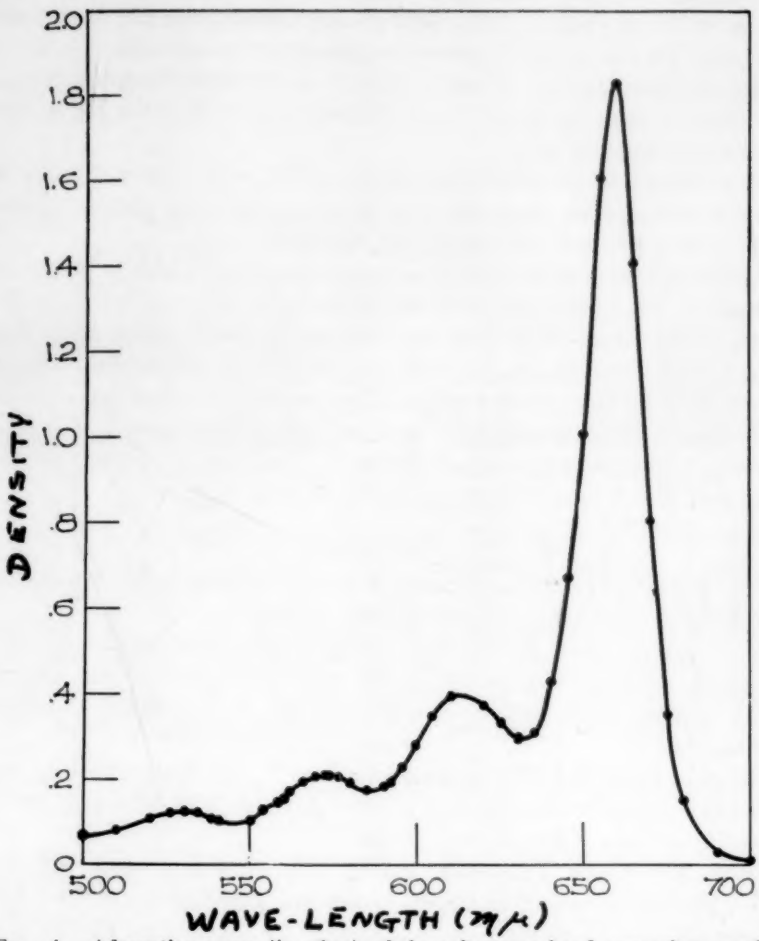

FIG. 4. Adsorption curve (in ether) of the only green band appearing on adsorption column after purification of extracted pigments from chlorina #2.

ina #1 has a chlorophyll content significantly higher than that of the normal plants. In #1, #6 and #8 the chlorotic condition apparently is a function of the quantity of chlorophyll present. Chlorina #3, #4 and #5 when compared to nonrelated "normals" show no significant differences from normal in total chlorophyll content. Here as in stock #3, the chlorotic condition (at least under these growing conditions) is apparently due

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not to a difference in chlorophyll content but to some other physiological factor. It is suggested that this factor might be an increase in the yellow pigments of the plant, or a morphological difference in the chloroplastids.

In spite of these differences in chlorophyll content, in all except stock #2 the ratio of a : b was within the normal range given by WILLSTÄTTER and STOLL (**19**).

The analysis of chlorina #2 indicates that chlorophyll b may be entirely lacking in these plants. This lack is corroborated by the fact that the absorption curves of the extracts are almost identical with a pure ether extract of chlorophyll a. Finally, the chromatographic method of separation failed to show the presence of chlorophyll b and gave only one green pigment, chlorophyll a.

In maize the seedlings lacking chlorophyll b were reported to be lethal (**3**). In barley these plants do grow and mature seeds, although they are not as vigorous and sturdy as normal varieties.

Of the various comparisons between chlorinas and normals, those within segregating lines (#1, #7 and #8) seem to be the most valid, since the genetic backgrounds of the two are more nearly alike. Even here, there is the objection that on the average, two-thirds of the normal sibs were heterozygous for the chlorina gene. The least valid comparisons are likely to be those in which the normals were used for comparisons with unrelated chlorinas. However, the large differences which were observed seem to indicate real differences. These are being studied further.

Summary

The content of chlorophyll a and b was spectroscopically determined in eight genetically different chlorina stocks in barley.

One chlorina, stock #2 (linkage group unknown), seemed to be entirely lacking in chlorophyll b as shown by spectrophotometric and chromatographic analysis. Its total chlorophyll content did not differ significantly from normal.

Chlorina stock #3 (fc) in linkage group 7, had a significantly higher chlorophyll a and b content than did the normals with which it was compared. The ratio of a to b was normal.

Stock #4 (Coast V; linkage group unknown) and stock #5 (Minn 84-7) with factor pair ff in group 1, are not significantly different from the normals in chlorophyll a and b content. Chlorosis must be due to some factor other than chlorophyll content.

Three chlorinas in the group investigated, *i.e.*, #1 (Trebi IV), #8 (Coast Dwarf) and #6 (Comfort II) were apparently chlorotic because of a low chlorophyll content, although the ratio of the two chlorophyll factors was normal.

One extreme chlorotic type, #7 (II26-361-4(23)), was completely lacking in chlorophyll.

One albino which happened to be segregating in a normal stock was completely lacking in chlorophyll but contained carotin.

I wish to acknowledge the invaluable advice and assistance of Dr. C. R. Burnham throughout the course of this study, and the advice and assistance of the late Dr. Rodney B. Harvey under whom this study was begun. I wish also to acknowledge the advice of Dr. C. S. French and Dr. Albert Frenkel on the preparation of chlorophyll extracts and Dr. Robert B. Griffith for his help in preparing the counter-current washing apparatus.

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MANGANESE INTERFERENCE IN THE ABSORPTION AND TRANSLOCATION OF RADIOACTIVE IRON (Fe^{59}) IN *ANANAS COMOSUS* (L.) MERR.[1]

C. P. SIDERIS[2]

(WITH ONE FIGURE)

Received April 24, 1949

Introduction

The interference of manganese with the availability of iron to pineapple plants has been explained by JOHNSON (**7**) and KELLEY (**9, 10**) as due to the oxidation of the latter by the former in soil and leaf tissues. Additional studies by SIDERIS, YOUNG and KRAUSS (**14**) and SIDERIS and YOUNG (**17, 20**) revealed that iron may be made unavailable in ample amounts to the leaf tissues after its precipitation in the exodermal root tissues at pH higher than 5.5, the latter resulting from the absorption by the roots of anions at higher rates than cations.

All evidence obtained so far indicates that the iron requirements of pineapple leaves are small; also, differences in the iron content of chlorotic and green leaves are, likewise, small.

Therefore, in order to avoid the uncertainty and difficulty of handling by ordinary chemical analyses the small amounts of iron absorbed by the roots at different periods and to eliminate the interference of preexisting amounts of this element in the tissues of the plants, the latter were grown in solution cultures supplied with iron containing a mixture of the radioactive isotopes Fe^{55} and Fe^{59}, as well as the other stable isotopes as stated by the U. S. A. E. C. (**28**).

Experimental procedure

CULTURAL METHODS

The crowns (vegetative organs born on the apex of fruits) of the clones Cayenne and 43–115 and the slips (vegetative organs born on the peduncle) of 10845 and 9429, sent by J. L. Collins to the writer, were suspended in nutrient solutions kept in 4-gallon enameled crocks which had been painted with asphalt paint to cover possible unenameled spots.

The nutrient solutions, constantly aerated during plant growth, contained 0.472 g. Ca $(NO_3)_2 \cdot 4H_2O$; 0.174 g. K_2SO_4; 0.080 g. NH_4NO_3; 0.246 g. $MgSO_4 \cdot 7H_2O$; 0.136 g. KH_2PO_4; 0.005 g. $ZnSO_4 \cdot 7H_2O$; 0.003 g. $Na_2B_4O_7 \cdot 10H_2O$ per liter.

[1] Published with the approval of the Director as Technical Paper No. 179 of the Pineapple Research Institute, University of Hawaii.

[2] The study was conducted in 1947 during the tenure of a Research Fellowship in the Division of Plant Nutrition of the University of California, at Berkeley, California.

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No iron was added to the nutrient solutions from August 23, the beginning day of the experiment, to October 23, the day of the receipt of radioactive iron.[3] However, none of the plants showed symptoms of chlorosis except clone 43–115, which was of less intense green than the other clones.

On October 23, 1947, two of the plants from a total of 12 per clone per treatment which were to be treated with radioactive Fe* were placed in 2-liter pyrex beakers, one per beaker and others in 4-gallon crocks, five per container. The pyrex beakers were shielded from direct sunlight by a cover of heavy black paper which was painted on the exposed side with aluminum paint to reflect light. The plants were suspended for subsequent growth in the nutrient solution contained in the pyrex beakers through holes in a concrete cover which was painted with asphalt paint, the latter employed to prevent absorption of the solution by the cover.

The sample of the radioactive iron which arrived as $FeCl_3$ in 7.9 ml. of HCl solution, with 455 mg. of Fe of an activity of 1.5 mc (millicurie) equivalent to 3.31 mc per gram and an intensity of radiation (unshielded) of 30 mr/hr at six inches. The total volume of 7.9 ml. of $FeCl_3$ in HCl was poured into a 150 ml. brown bottle containing 20 ml. of 10% metaphosphoric acid (HPO_3) to which was added 44.1 ml. of water to raise the total volume to 72 ml. Metaphosphoric acid was added to convert ferric iron to the metaphosphate salt which is very soluble, Fe not precipitable within a wide range of pH. The mixture equivalent to 6.3 mg. of Fe per ml. of solution was employed as a stock solution of Fe* for the nutrient solutions.

The plants grown in the 2-liter pyrex beakers were supplied with 20 γ of Fe, and either with 100 γ of manganese as $MnSO_4 \cdot 4H_2O$, or without. Those grown in the 4-gallon crocks were supplied with 4 γ of Fe, but not with manganese.

Analytical methods

Sections 3, 4 and the lower portions of 5 of the chlorophyllous regions of the mature (C) and active (D) leaves and roots were selected for measurements of tissue radioactivity on the basis of previous studies (**14, 17, 20**) which showed that iron accumulations were greater in these than in other tissues of the leaves. The extreme terminal sections of the leaves, although richer in iron, were discarded because of the onset of senescence much earlier in these than in other tissues.

Depending on the intensity of the radioactivity of the tissues, the fresh weights used for a single determination ranged from one to 25 grams. Such tissues, placed in porcelain evaporating dishes and dried to obtain dry weight values, were then ashed in an oven. The ash, dissolved in 5 ml. of 1:1 HCl solution, were transferred to conical 15 ml. centrifuge tubes

[3] This isotope was obtained from the Isotope Division of the U. S. Atomic Energy Commission at Oak Ridge, Tenn., and contained Fe^{55} and Fe^{59}, the latter being the more active fraction, will be designated in subsequent references as Fe*.

where sufficient 27% NH_4OH was added to make the solution alkaline and precipitate iron. The solution, after standing for 30 minutes, was centrifuged at 0° C. The supernatant liquid was discarded and the precipitate, after suspension in 2 ml. of acetone, was transferred by a pipette with a wide opening dropwise on to a metal disc of tinned iron 2.5 cm. in diameter kept on a hot plate at very low heat. The use of acetone for suspension of the precipitate is for the purpose of rapid evaporation of the mixture. In case of an incomplete removal of the precipitate from the centrifuge tube and from the pipette, both tube and pipette are treated with a weak solution of HCl placed in the tube, the iron is again reprecipitated with NH_4OH, centrifuged, and the precipitate transferred to the metal disc as before.

Great care must be exercised to avoid excessive heating of the metallic disc because of the danger of losing the precipitate by spattering, which is caused by a rapid vaporization of the solution. The use of infrared lamps for drying such precipitates should be preferred to the hot plate method. However, a sheet of asbestos placed over the entire area of the hot plate regulated satisfactorily the heat radiations and the evaporation of the solution. In case of very weak adherence of the dry precipitate to the metallic disc, a drop or more of 5% sucrose added carefully with a pipette without touching the precipitate will prevent possible losses.

The precipitates on metallic discs are ready for measurement of their radioactivity with a Geiger-Muller counter. The thickness of the mica window of the tube best suited for such measurements and the distance between the radioactive substance and the tube depend on the intensity and variety of the radioactive emanations. The thickness of the G-M window was approximately 3 mg/cm^2, presumably well adapted for the measurement of the emanations from Fe^{59} but not from Fe^{55}.

Due to the much stronger iron content of the root than leaf tissues, the isolated iron from the leaf samples was placed approximately at 0.5 inch from the G-M window and that of the root samples 2.0 inches. A radium standard placed at the two distances gave 300 and 60 counts per minute for the 0.5 and 2.0 inch distances, respectively. The background radiations varied slightly for one month the entire period of the measurements, ranging from seven to nine cts/m. The total ash content of the leaves, consisting mostly of K, Ca, Mg and P-salts, was approximately five times as great as that of the roots. However, the relationship for iron was reversed; this element was approximately 20 times as great in the roots as in the leaves, *i.e.*, 3.5 and 70.0 γ per cm^2 of the metallic disc for the leaves and roots, respectively. Absorption of Fe^* by sample thickness, not determined by standardized quantities of Fe^*, was presumably small, due to the small amounts of total iron contained in the samples.

Cobalt, unavailable at the time of these measurements in the laboratory, could have served as a better standard than radium, which was available, because the latter emits mostly alpha particles and the former beta and

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gamma radiations which, also, are emitted by Fe*. However, for obtaining a factor of radiation intensity at different distances between the G-M counter and the experimental material, it was thought that the results obtained with radium, which is used as an auxiliary unit for gamma ray emitters (**8**), could not deviate greatly from those with Co^{60} or Fe^{59}.

Still a better method would have been the use of Fe* standards of different intensities of radioactivity.

The number of counts per minute per 0.01 mg of Fe in solution, 25 days after its receipt from Oak Ridge, at 0.5 inch from the G-M tube, was approximately 100. The same distance from the G-M tube was used for measuring the radioactivity of nearly all the leaf but not the root samples.

The distance for the roots, approximately 2.0 inches, in table II, was converted to the 0.5 inch distance by use of the factor obtained with the radium standard. However, the counts for both distances are reported in this table. It is possible that the converted values in the roots deviate to some extent due to the difference in the kinds of radiations emitted by Fe* and Ra*.

The radiation counts of the leaves all obtained at 0.5 inch distance are reported as such in all tables without conversion by the factor of the Ra standard.

Possible deviations between the leaves of different clones or between the plus Mn and minus Mn cultures of these clones were eliminated by the use of identical tissue weights and distances from the G-M tube for the measurement of their radiations.

The radioactivity of the background was determined before and after each determination of the radioactivity of the sample. The latter was measured at three successive intervals and the average number of counts per minute minus the average number of the background counts, which ranged from seven to nine per minute, divided by the number of grams of fresh weight of the plant tissues, gave the values reported in the various tables as cts/m/g (counts per minute per gram).

The same conditions of uniformity of tissue weights were adopted for the roots, except the distance from the G-M counter, which was 2.0 inches for all root measurements.

Thus in table II the values under roots, cts/m/g, indicate those actually obtained at the 2.0 inch distance and those calculated at the 0.5 inch distance by the factor of the Ra standard.

Fractionation of cellular components

The possibility of a greater association of iron with certain cellular components than with others suggested the idea of fractionating leaf tissues into classes characterized by certain chemical and physical properties. For this purpose, leaf tissues mixed with a solution of potassium hydroxide were macerated in a Waring Blendor. The resulting pulpy mixture was

separated into solid and liquid portions and the proteinaceous matter recovered from the latter by precipitation with metaphosphoric acid. Details of this procedure are, as follows:

Fifty gm. of leaf tissues were suspended in 100 ml. of 0.05N KOH in a Waring Blendor and macerated for one minute. At the end of this period, the acidity of the mixture was examined and more KOH was added to raise the pH above 7.0 but below 8.0 and the maceration was continued for 10 minutes longer. At the end of this period, the mixture was transferred to the coarse (not nappy) side of a piece of Canton flannel held in a 6-inch funnel and the liquid was extracted therefrom by manual pressure. The residue in the flannel was wetted with 0.05N KOH and squeezed four times in succession until a final volume of 250 ml. was obtained.

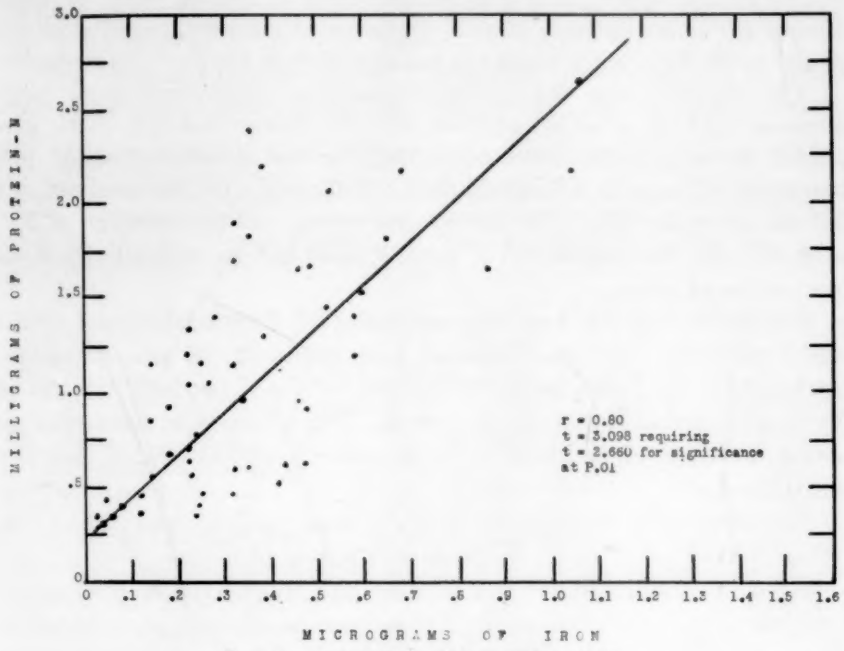

FIG. 1. Correlation of iron and protein.

The residue in the flannel, composed mostly of cell wall material admixed with some proteinaceous matter, fibers and other insoluble substances, was carefully removed with a spatula, weighed and divided into two equal portions, one of these used for the determination of proteinaceous nitrogen by the Kjeldahl method, and the other for dry weight with subsequent measurement of its radioactivity after ashing by the method as mentioned above. The latter fraction should have been washed with metaphosphoric acid to remove possible traces of precipitated ferric iron after treatment with KOH, but due to oversight in the beginning and lack of ample time in the end of this study, such treatment was omitted.

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The liquid fraction was centrifuged in 250 ml. centrifuge bottles to remove starch and traces of fibrous and parenchymatous tissues and with some chloroplastic matter. The supernatant liquid was carefully removed, its volume determined and then divided into two equal parts which were placed in separate 250 ml. centrifuge bottles, one intended for the determination of nitrogen and the other, after ashing, for radioactivity in the proteinaceous matter. These liquids after treatment with appropriate volumes of 10% metaphosphoric acid to cause complete precipitation of the proteinaceous-colloidal matter in suspension were centrifuged for 20 minutes in a centrifuge kept at 0° C. The supernatant colorless or slightly greenish yellow but clear liquid was decanted carefully in a flask and then the proteinaceous precipitate in the centrifuge bottles was treated with 50 ml. of 1% metaphosphoric acid and recentrifuged to remove traces of water soluble Fe^{59}. At the end of this operation, the supernatant liquid was placed in the flask which contained the liquids from the previous operation.

The precipitates in the bottles, loosened by gentle centrifugal movements of the hand after the addition of 5 ml. or more of 0.01N KOH, were poured in appropriate containers; that for the determination of proteinaceous nitrogen in a Kjeldahl flask and the other for radioactivity in a 250 ml. porcelain dish. The latter, after ashing and precipitation of Fe^{59} with NH_4OH, was transferred to metallic discs and its radioactivity measured as stated above.

The liquid fraction from the separation of the proteinaceous matter was divided into two equal volumes, each placed in 250 ml. centrifuged bottles and treated with acetone in the ratio of one to two that is, 80 ml. of the liquid fraction of 160 ml. of acetone. The mixtures in the centrifuge bottles, were placed for three hours in a refrigerator at –23° C and then centrifuged at 0° C for 20 minutes.

The supernatant liquids were carefully decanted into clean 250 ml. centrifuge bottles while the precipitates, with strong peroxidase activity, were dissolved in 10 ml. 0.01N KOH solution and transferred to appropriate containers for the determination of proteinaceous nitrogen and radioactivity as mentioned above. The supernatant liquids from this operation, treated in the 250 ml. centrifuge bottles with a few ml. of 27% NH_4OH to precipitate Fe^{59}, were centrifuged. The resulting supernatant liquid was discarded and the precipitate, dissolved in 5–10 ml. of 1:1 HCl, was transferred to 15 ml. conical centrifuge tubes, where Fe^{59} was precipitated out with a few ml. of 27% NH_4OH, then centrifuged and the precipitate transferred to metallic discs, as stated above.

The various fractions obtained by the above procedure, although not pure chemical entities but mixtures of related substances, are designated in the text as cellulose-lignin (cell wall and fibers), protein, peroxidase and inorganic Fe^{59} fractions.

Plant growth

During the 2-month period of growth (August 23 to October 23) before the addition of Fe^{59} to the cultures, it became obvious that the rate of growth as measured by the length, width and number of leaves per plant was different for the various clones. Also, the number and length of the roots and other related morphological features such as thickness, lateral roots and root hairs, likewise, differed in the various clones.

Clone 43–115 produced considerably longer leaves, and fewer roots than Cayenne. Also, the roots of 43–115 were thinner and carried fewer laterals than Cayenne, which may account in part for certain differences in the absorption of nutrient elements between these clones, as reported below. Clones 10845 and 9429 produced fewer leaves and roots than Cayenne but the roots in the former were equipped with more but very slender laterals than in the latter.

Plant weights, calculated on the new growth (new tissues) of 10 leaves attained between August 23 and November 3, 1947, are reported in table I.

TABLE I

Weights of the new growth of ten leaves produced by different clones grown in nutrient solutions without added iron at Berkeley, California, from August 23 to November 3, 1947

Clones	Leaf weights (fresh)	
	gms.	%*
Cayenne	186.0	98.5
43–115	188.2	100.0
10845	112.5	59.8
9429	97.5	51.9

* Calculated as per cent. of 188.2 of clone 43–115.

The values were obtained by weighing 10 of the mature (C) and active (D) groups from which small terminal sections representing tissues from growth before August 23 had been severed.

The data showing very great differences between the weights of Cayenne and 43–115, on the one side, and of 10845 and 9429 on the other, may have been responsible for the different rates of absorption of mineral elements from the nutrient solutions or their dilution in plant tissues.

Radioactivity of plant tissues

The data in table II, indicating the radioactivity of Fe^{59} in the leaf and root tissues of different cultures, are reported as counts (ct) per minute (m) per gram (g) of fresh tissue (**8**).

All data are based on 10 grams of fresh tissue from two leaves per plant and two roots per plant, the latter weighing from one to two grams after removal with blotting paper of the superficial water of the nutrient solution.

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The data in table II, showing different amounts of Fe^{59} in the roots and leaves of the various clones, may suggest that the rate of growth of such clones, together with certain differences in the morphology of their roots might have been the contributing factors to the differences in iron absorption, translocation and concentration in the tissues. The differences in the amounts of Fe^{59}, as measured by the radioactivity of the tissues, between leaves and roots in the same clone, in favor of the roots, were very great. They suggest that most Fe^{59} in the roots was not absorbed into the tissues but presumably precipitated on the exodermal layer of cells, a condition also observed in previous studies (**14, 17, 20**). In the cultures supplied with 100 γ of Mn, similar differences were greater, suggesting that Mn had considerably increased the deposition of Fe^{59} on the exodermal tissues of the roots.

TABLE II

RADIOACTIVITY OF Fe* AS CTS/M/G IN COMPOSITE SAMPLES OF NO. 4 SECTIONS OF THE MATURE (C) AND ACTIVE (D) LEAVES OR IN THE ROOTS MEASURED AT 0.5 AND 2.0 INCH DISTANCES, RESPECTIVELY, FOR THE CAYENNE, 43–115, 10845 AND 9429 PINEAPPLE CLONES GROWN FOR 15 DAYS (OCTOBER 23 TO NOVEMBER 6, 1947) IN SOLUTION CULTURES SUPPLIED WITH 20 γ OF Fe AND EITHER WITH OR WITHOUT 100 γ OF Mn.

CLONES	TREATMENT		LEAVES	ROOTS		CTS/M/G ROOTS/LEAVES
	Fe γ	Mn γ	0.5" *cts/m/g*	2.0" *cts/m/g*	0.5"* *cts/m/g*	
Cayenne	20	0	58	245	1225	21
43–115	20	0	12	358	1790	149
10845	20	0	22	558	2790	127
9429	20	0	41	455	2275	55
Cayenne	20	100	14	490	2450	175
43-115	20	100	9	503	2515	280
10845	20	100	14	630	3150	225
9429	20	100	25	654	3270	131

* Calculated: The values of cts/m/g obtained at the 2 inch distance were multiplied by five, the ratio obtained by the radium standard for the two distances.

The figures in the last column of table II, indicating root-iron to leaf-iron ratios, reveal that Fe^{59} was more in excess in the roots than in the leaves of the various clones, ranging from 21 to 149 times for the minus-Mn cultures and from 13 to 280 times for the plus-Mn cultures.

Somewhat similar data, obtained for the same clones in plus-Mn and minus-Mn cultures grown for 20 and 30 day periods, are reported in table III.

The data in table II show that considerably less iron was translocated from the roots to the leaves of the plus-Mn than minus-Mn cultures. Except for the differences in the periods of absorption of Fe* by roots from the nutrient solutions, the data in tables II and III are almost the same with respect to the influence of manganese on the iron content of the leaves.

A radioautograph, not presented here, showed that Fe* was distributed throughout the entire leaf area although in somewhat greater amounts toward the terminal than basal regions. A semicircular zone about 2.5 cm. from the tip of the leaf, indicating the line of demarkation between the tissues of the old and new growth, accumulated more iron than other areas. Although the area of the old growth between the tip and line of demarkation contained some iron, the radioautograph did not reveal any greater concentrations in the fibrovascular than in the mesophyllic tissues as shown occasionally in chlorotic leaves by the formation of more chlorophyll along the leaf veins, following iron absorption through the roots, than in the nearby mesophyllic tissues. It is possible that the even distribution of the iron radiations in the leaf tissues resulted from the great thickness of the leaf, about 3 mm. which interfered with their outward penetration and recording on photographic film. Such radiations were highly intensified at the edges of the leaf, where the thickness was approximately 1 mm.

TABLE III

RADIOACTIVITY OF Fe* AS CTS/M/G IN THE CHLOROPHYLLOUS SECTIONS OF THE LEAVES OF CAYENNE, 43–115, 10845 AND 9429 PINEAPPLE CLONES GROWN IN SOLUTION CULTURES FOR 20 OR 30 DAYS (OCTOBER 23 TO NOVEMBER 23, OR OCTOBER 23 TO NOVEMBER 23, 1947) SUPPLIED WITH 20 γ OF Fe AND EITHER WITH OR WITHOUT 100 γ OF Mn.

PINEAPPLE CLONES	20-days			30 days		
	CULTURES Fe = 20		RATIOS Mn = 0 γ: Mn = 100 γ	CULTURES Fe = 20		RATIOS Mn = 0 γ: Mn = 100 γ
	MINUS Mn	PLUS Mn		MINUS Mn	PLUS Mn	
	cts/m/g			*cts/m/g*		
Cayenne	121	lost	.....	118	44	2.68
43-115	46	20	2.30	51	38	1.34
10845	32	15	2.13	.....	.....	.....
9429	55	38	1.45	.....	.....	.....

Previous studies (**14, 15, 16, 17, 18, 19, 20, 21, 22**) have indicated that iron, chlorophyll and protein gradients increased from the basal to the terminal sections of leaves, suggesting presumably some association between iron and either chlorophyll or protein, or both. Because of the existence of various metallo-proteins (metalloporphyrins) in plants and animals, certain analyses were made to obtain pertinent information, which is reported in table IV.

The data in table IV show that the radioactivity in the protein and peroxidase fractions of the 23-day plants differed considerably from that of the 30-day plants in the Cayenne clone. Lack of time prevented the writer from repeating the analyses to detect the source of disagreement in the peroxidase fraction.

However, the data in the 23-day cultures show considerably greater

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amounts of Fe^{59} in the various cellular components for the Cayenne than for the 43–115 clone, which conform with the results in table III. The protein fraction, which may be considered the least contaminated with inorganic Fe^{59}, possessed greater radioactivity than the cellulosic-ligneous fraction, although the latter was four to five times greater in weight than the former. The high values of the peroxidase fraction in the 23-day plants cannot be explained.

The radioactivity of the 30-day plants, although varying slightly from the 23-day plants, shows, except for the peroxidase fraction, the same relative distribution of iron in the various cellular components. The nitrogen values, intended to reveal the distribution of proteinaceous matter in the various fractions, show that the greatest portion of Fe^{59} was in the protein; other fractions contained much smaller amounts.

TABLE IV

RADIOACTIVITY OF Fe* IN VARIOUS CELLULAR COMPONENTS AS CTS/M/G OF FRESH LEAF TISSUE OR OF DRY CELLULAR COMPONENTS AND MILLIGRAMS OF PROTEIN NITROGEN PER GRAM OF FRESH LEAF TISSUE, IN CAYENNE AND 43–115 PINEAPPLE CLONES GROWN IN SOLUTION CULTURES WITH 4 γ OF Fe FOR 23 AND 30 DAYS

Cellular components	23 days Mature (C) leaves		30 days—Cayenne Active (D) leaves				
	Cayenne fr. wt.	43-115 fr. wt.	Fresh tissues		Dry tissues		
					Weight		cts/m/g
	cts/m/g	*cts/m/g*	*cts/m/g*	*N mg/g*	*gm.*	*%*	*gms.*
Cell. × Lign*	16.50	2.33	17.0	0.266	0.355	81	48.0
Protein†	16.80	3.80	11.2	1.410	0.083	19	135.0
Peroxidase	16.80**	6.70	4.0	0.112	.....	.....	.....
Inorganic	.....	.....	1.0	.....	.....	.....	.....

* Cell. × Lign. = Cellulosic and Ligneous (cell walls and fibers).
** Peroxidase precipitate not ashed but dried and radioactivity measured.
† Impure preparation.

The Fe^{59} content, as indicated by the radioactivity in the various fractions, does not correlate positively with the protein-N of the fractions when calculated per unit of fresh leaf tissue. However, if such radioactivity is calculated per unit (gram weight) of their respective cellular components, as in the last column in table IV, it increases directly with the amounts of the proteinaceous fraction, suggesting that iron is associated, although not with the catholic proteinaceous content of the cell, at least with a certain small section of this fraction.

The question has often been raised (**2, 7, 9, 10, 26, 27**) whether iron is rendered unavailable by oxidation with subsequent precipitation by manganese in the nutrient solution or soil before absorption by plant roots or after absorption. In order to obtain information on this subject, 5 ml. of the nutrient solution from the plus-Mn and minus-Mn cultures, exposed to

the action of plant roots for 23 days at pH 4.5–6.0, were placed in containers which were examined under the Geiger-Muller counter for radioactivity. The results of these findings, indicating for the Cayenne 445 cts/m/g in the minus-Mn and 411 cts/m/g in the plus-Mn cultures, and for clone 43–115, 452 cts/m/g in the minus-Mn and 460 cts/m/g in the plus-Mn cultures, reveal no significant differences between the minus-Mn and plus-Mn cultures.

The data might indicate that there were hardly sufficient changes to make certain that precipitation of Fe by Mn had taken place in the nutrient solution. Although the conditions were unsuitable for the oxidation and subsequent precipitation of Fe^{59} in solution cultures supplied with metaphosphate as the sole source of PO_4 and at acidities ranging mostly from pH 4.0 to 5.5, nevertheless, depositions of Fe^{59} took place on the exodermal root tissues more so in cultures supplied with Mn than without. Therefore, the results suggest that certain conditions on the root surface, not yet satisfactorily explainable, develop in the absence as well as in the presence of Mn during the absorption of iron which are primarily responsible for the oxidation and precipitation of iron. The presence of Mn increases the amounts of Fe precipitation. However, such precipitated iron may be made slowly available to plants by the action of acids resulting from a greater rate of absorption of cations than anions or by reducing agents (**14, 17, 20**).

Discussion

The results presented above indicate that in nutrient cultures supplied with or without manganese, iron was deposited in great amounts in the exodermal tissues of pineapple roots, some of which was translocated to the leaves but the greatest portion remained insoluble and consequently unavailable. In cultures with high concentrations of Mn (100 γ), the amounts of iron deposited in the exodermal root tissues increased and those translocated to the leaves decreased.

Because of the deposition of great amounts of iron in the exodermal tissues of roots in the absence of Mn, which may be increased considerably in its presence, the causes for the precipitation and deposition of iron cannot be alone ascribed to the action of Mn. Such causes, inherent in the exodermal tissues of the roots, are only augmented in the presence of Mn.

Previous studies (**14, 17, 20**) showed that high pH values generated by a greater rate of absorption by roots of anions than cations (NO_3 vs. K or Ca) contributed as much to the precipitation and deposition of Fe in the exodermal tissues of roots and to the low amounts in the leaves as high amounts of Mn in the nutrient solution.

Possible mechanisms for explaining the deposition of iron in the exodermal tissues of roots grown in solution cultures supplied with or without Mn may be attributed to (a) the alkalinity of the products of hydrolysis of

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bicarbonate ions (HCO_3) formed from CO_2 by respiring roots, and (b) the oxidation of iron by manganese according to the reaction:

$$Fe^{++} + Mn^{+++} \rightleftharpoons Fe^{+++} + Mn^{++}$$

Both reactions, resulting in the oxidation of Fe^{++} to Fe^{+++}, are possible in soils of higher pH than 5.5 and in the exodermal tissues of roots but not as much in the chlorophyllous tissues of leaves with high acidity (pH 3.4–4.6) and reducing agents such as ascorbic acid (**15, 18, 21**).

The production of chlorosis in soybean plants only by certain iron and manganese combinations and not by others caused SOMERS and SHIVE (**25**) to assume that good growth and development of plants free from pathological symptoms depended on a two to one ratio of iron to manganese within the plant tissues and in the nutrient solution, and that ratios with values higher than two produced iron toxicity, and lower than two, manganese toxicity. A similar view has also been shared by TWYMAN (**27**). However, BENNETT (**1**) found in tomato plants that ratios of iron to manganese ranging from 1.6 to 0.1, with maximum Fe at 50 γ and Mn at 80 γ, and with minimum Fe at 5 γ and Mn at 5 γ produced green leaves, whereas similar ratios ranging from 0.08 to 0.01, with maximum Fe at 8 γ and Mn at 180 γ, and with minimum Fe at 0.5 γ and Mn at 50 γ produced chlorotic plants. Unpublished data of the writer have shown that iron to manganese ratios higher than 0.01, with maximum Fe at 5 γ and Mn at 50 γ, and with minimum Fe at 0.5 γ and Mn at 5 γ produced green plants, but similar ratios of 0.001 with Fe at 0.05 γ and Mn at 50 γ produced chlorotic plants.

The above comparisons show that different plants may require different iron-manganese ratios because according to previous studies (**14, 17, 20**), the absorption and translocation of iron may depend on the acidity of the exodermal root tissues, which is affected by the ratio of cations to anions entering the roots simiultaneously. Moreover, the rate of utilization of either Fe or Mn in the tissues for the synthesis of metalloproteins may determine the rate of translocation of these elements from roots to leaves. The toxicity theory (**25, 27**) at concentrations 50 γ of Mn or 5 γ Fe, although possible, cannot be easily understood, since the writer witnessed pineapple plants grown by J. P. Bennett in solution cultures supplied with 300 γ of Mn and some even with higher amounts, unless such plants were not absorbing Mn at the rate of supply.

The results in table IV, showing a certain degree of association of iron to protein, and previous studies (**14, 15, 16**), summarized in figure 1, indicating positive correlation between iron and protein ($r = 0.80$ and $t = 3.098$, requiring for significance $t = 2.660$ at P.01), suggest that iron may be in stoichiometric relationship with a certain section but not with the entire proteinaceous fraction of the cell. However, with future improvements in the subdivision of the proteinaceous fraction, the iron-containing section may be isolated and identified.

Protein, chlorophyll and iron relationships in a variegated variety of *Ananas comosus* (L.) Merr. are reported in table V.

The data indicate that with greater amounts of chlorophyll in the green than chlorotic areas of the same leaves both protein and iron increased, but not in stoichiometric proportions.

The occurrence of iron in the chloroplasts of various plants has been reported by MOOR (**13**). Similar findings were reported for the chloroplasts of *Zea mays* and *Hordeum distichum* by GRIESSMEYER (**3**), for spinach by LIEBICH (**11**) and Claytonia by HILL and LEHMAN (**5**). JACOBSON (**6**) found in tobacco that from 65 to 85% of the total leaf iron was in the chloroplasts. Also, MOMMAERTS (**12**) and JACOBSON (**6**) found iron in the purified chlorophyll protein complex substances of corn and tobacco leaves, which results are in agreement with the data in table V for pineapple leaves.

These results and those of others indicating certain relationships between chlorophyll, protein and iron suggest that all three substances must bear a stoichiometric relationship in a certain section of the proteinaceous complex in the chloroplast, the identity of which has not yet been definitely established.

TABLE V

CHLOROPHYLL, PROTEIN-N AND IRON IN GREEN AND CHLOROTIC AREAS OF THE LEAVES OF A VARIEGATED STRAIN OF *Ananas comosus* (L.) MERR.

CELLULAR COMPONENTS OF DRIED TISSUES	LEAF AREAS		RATIO
	GREEN MG/G	CHLOROTIC MG/G	GREEN: CHLOR.
Chlorophyll	2.76	0.38	7.25
Protein	88.00	57.80	1.47
Iron	0.0095	0.0041	2.32

Summary

The following results were obtained from experiments on the absorption and translocation of radioactive iron (Fe^{59}) in pineapple plants grown in nutrient solutions supplied with 20 γ or 4 γ of Fe^{59}, and either with 100 γ or without manganese.

1. Most iron removed from the nutrient solution was deposited in the roots, presumably in the exodermal tissues, more so in the cultures supplied with than without manganese.

2. The amounts of translocated iron from the roots to the leaves was considerably lower in the cultures supplied with than without manganese.

3. No precipitation of iron could be detected with certainty in the nutrient solutions containing manganese as a result of the reaction $Fe^{++} + Mn^{+++} \rightleftharpoons Fe^{+++} + Mn^{++}$.

4. Considerable amounts of the translocated iron remained in the proteinaceous matter of the cells and others in the cell walls and substances with peroxidase activity.

5. The data suggest that iron may occur in combination with some proteinaceous fraction, presumably an enzyme, which activates the formation of certain other proteins intimately related with chlorophyll.

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THE GROWTH AND METABOLISM OF EXCISED ROOTS IN CULTURE. I. THE MEASUREMENT OF GROWTH AND THE ROLE OF CERTAIN VITAMINS

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(WITH THREE FIGURES)

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It has long been recognized that one of the most essential needs of investigators of problems of growth and development in plants is experimental material in which growth and development can be studied in a rigidly controlled environment. Methods for the culture of parts of plants in nutrient media under sterile conditions provide, in a measure, a means of controlling the environment. Successful isolated root culture experiments have been conducted by Kotte (**6, 7**), Robbins (**8**), White (**16**), Addicott and Bonner (**1**) and Bonner and Devirian (**3**) on more than 20 species of dicotyledonous plants. Much less success has characterized attempts to culture roots of monocotyledonous plants (Whaley and Long, **15**). In general, the investigations have been concerned either with improvement of the techniques or with determining the general picture of physiological requirements of roots.

Bonner and Devirian (**3**) and Bonner (**4**) have shown that roots of different species vary appreciably as to the nutrients with which they must be supplied for satisfactory growth in culture. Robbins (**13**) and Whaley and Long (**15**) have shown that roots of different lines of tomato differ particularly in vitamin requirements. The results do not make it clear as to whether the differences in response to added vitamins represent differences in ability of the roots to synthesize these substances for themselves or differences in ability to utilize the supplements. It can be anticipated, however, that if such differences exist among lines within a given species they may play some part in the development of general metabolic differences. It should be possible to study the inheritance patterns of these differences by utilization of root culture techniques in combination with a hybridization program to produce progeny in which these same characteristics can be investigated. The present study has been aimed at detecting the existence of line differences in roots of the tomato, *Lycopersicon esculentum* Mill. and related species with respect to the ability of the roots to synthesize and/or utilize thiamin, pyridoxine and niacin; at evaluating the significance of such differences; and ultimately at studying the inheritance of these differences.

Materials and methods

The tomato roots used were those of the University of Texas laboratory line number 151. Seeds of this particular line, inbred through six generations, were kindly furnished by Dr. Leroy Powers of the United States Horticultural Station in Cheyenne, Wyoming.

Seeds were sterilized in 1% bromine water, washed repeatedly in sterile water, and germinated on 1% sterile agar. When the roots had emerged sufficiently, a one-centimeter section of each selected root tip was aseptically transferred to a 125 ml. Erlenmeyer flask containing 50 ml. of nutrient solution. After two weeks, one flask was selected on the basis of good root growth, and all the others were discarded. The root selected was removed from the flask to a dish containing sterile nutrient solution and a one-centimeter apical piece was cut from each lateral root. Each of these lateral root pieces was then transferred to a nutrient-containing flask where it was allowed to grow for another two-week period. In this manner a stock supply of roots was built up. Barring the possibility of mutation during the course of the experiment (there was no suggestion of such mutation), it can be assumed that the roots used were all completely uniform genetically.

The nutrient used was the one now generally referred to as White's (**22**) mineral solution. It contains per liter: $MgSO_4$—360.0 mg., $Ca(NO_3)_2$—200.0 mg., Na_2SO_4—200.0 mg., KNO_3—80.0 mg., KCl—65.0 mg., $NaH_2PO_4H_2O$—16.5 mg., $Fe_2(So_4)_3$—2.5 mg., $MnSO_4$—4.5 mg., $ZnSO_4$—1.5 mg., H_3BO_3—1.5 mg., KI—0.75 mg., and 20000.0 mg. of sucrose. Three mg. of glycine per liter were also added on the basis of White's (**20**) recommendation with reference to increased growth of tomato roots in solutions containing glycine, although, as has been suggested, it is to be doubted that the glycine is an essential addition. To the solutions in which the root clones were built up there were added, per liter of the solution, 0.1 mg. of thiamin, 0.1 mg. of pyridoxine, and 0.5 mg. of niacin. Building up of the stocks was thus accomplished in what is referred to below as the full vitamin supplement medium, containing all three of the vitamin supplements studied. In the series of experiments this full vitamin supplement medium and seven variations of it were used. These were: (1) the complete nutrient solution consisting of mineral salts, sucrose, and glycine, and supplemented with thiamin, pyridoxine, and niacin, (2) the solution complete as above, but without the vitamin supplements, (3) the solution complete as above plus thiamin only (niacin and pyridoxine deficient), (4) the solution complete as above, plus thiamin and pyridoxine (niacin deficient), (5) the solution complete as above, plus pyridoxine (niacin and thiamin deficient), (6) the solution complete as above, plus niacin and pyridoxine (thiamin deficient), (7) the solution complete as above, plus niacin (thiamin and pyridoxine deficient), (8) the solution complete as above, plus niacin and thiamin (pyridoxine deficient).

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When the number of root tips in the stock cultures was sufficient to provide a complete experimental set the roots were removed from the flasks and lateral root tip segments were removed from each of the roots for transplanting into the solutions in which their growth was to be evaluated. To preclude the possibility of transferring vitamins from the stock culture solutions into the culture media in which final growth measurements were to be made, a "weaning" period was provided. Root tip segments were transferred from the stock culture solutions to flasks containing the nutrient of the same composition as that in which final determinations were to be made. They were allowed to grow in these flasks for a period of ten days; then subcultures of lateral root tip segments were made from these roots and transferred to new flasks of the same solutions. Thus, uniform one-centimeter lateral root tip segments were introduced into 80 flasks of each of the experimental solutions. All cultures were maintained at a constant temperature of 28° C and were exposed to weak, diffuse light from a mixture of incandescent bulbs and fluorescent tubes.

Samples were taken at seven day intervals. At each sampling eight cultures were selected from each treatment for volume and weight determinations, two were set aside for histological studies, and two were reserved for respiration measurements. In selecting the cultures those showing little or no growth, probably as the result of injury during transfer, or those aberrant in any other manner were discarded, as were all visibly contaminated cultures. The present paper is concerned with the measurement of growth as determined by volume, fresh weight and dry weight. The results of the respiration measurements and those of the histological studies will be reported in separate papers.

Earlier attempts at evaluating growth of root cultures have been confined to measurements of length and weight determinations. BONNER and DEVIRIAN (**3**) measured the length of the main root during the first week after the setting up of cultures. In many root lines there is little or no branching. In his earlier papers on wheat (**16, 17, 18**), White measured growth by determining length increase of the main root axis plus the length of all lateral roots; in other words, a figure of total root length. In the remainder of his papers (**19** *et seq.*) White has used the length of the central axis of the root as a measure of growth. FIEDLER (**5**) has used both length and dry weight as measures of growth of corn roots in culture. ROBBINS and his co-workers (**9, 10, 11, 12, 13, 14**) and WHALEY and LONG (**15**) have based growth determinations principally on dry weight measurements. While length and dry weight measurements are quite satisfactory for comparative studies within a single strain of roots, or for studies in which various strains of roots are compared over relatively short periods of time, it is essential to have a more complete measure of the various aspects of growth if this technique of root culture is utilized for the study of relatively minor physiological differences in roots of different genetic consti-

tutions, because the patterns of growth differ both genetically and in response to different culture conditions.

With this fact in mind, volume increase, fresh weight increase, and dry weight increase have been determined with the thought that these three figures would give a fairly comprehensive picture of mass increase and actual protoplasmic syntheses, and that the results could be evaluated against those obtained in the respiration and histological studies. The measurement of volume in these small roots was made by the use of an especially designed volumeter. This apparatus (figure 1) operates on the

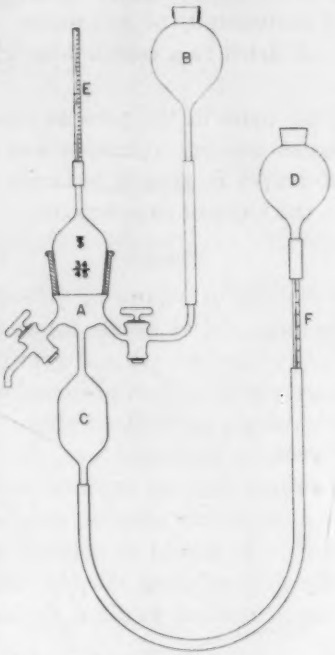

FIG. 1. Volumeter with which the volumes of roots were determined. The apparatus consists of two water reservoirs A and B, two mercury reservoirs C and D, and two pipettes E and F which are calibrated in 0.001 ml. divisions. In operation, the level of the mercury in pipette F is recorded when reservoir A is filled with water until the water overflow extends to the zero volume in pipette E. The water in reservoir A is then transferred to reservoir C by lowering bulb D. The tissue is placed in reservoir A and the water readmitted to this chamber by raising bulb D. When the mercury leveling bulb is so adjusted that the mercury level in pipette F is the same as the initially recorded value, the increased volume in pipette E is equal to the volume of the plant tissue. Water reservoir B and the two stopcocks are used to adjust the water levels to zero and to aid in cleaning reservoir A.

displacement principle. While this volumeter cannot be used for a series of rapid measurements, it has been developed to the point of sufficient accuracy to allow for the detection of very small differences in volume. Before it was utilized on this experimental material, a large number of indi-

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vidual roots were measured repeatedly and the results compared with average figures determined from the measurement of several roots at one time. It was established that the variation within individual samples was significantly less than the differences between samples. After this fact had been determined, roots were measured in groups rather than individually and an average root volume was calculated. When roots became too large for measurement in this apparatus, they were measured in another volumeter which is a simpler adaptation of the displacement principle.

After the root volume had been determined, the same roots used for the volume determinations were blotted lightly with filter paper to remove excess water and weighed individually or in groups. They were then placed in an oven at 105° C and dried to a constant weight for a dry weight determination.

Inasmuch as all of the roots in the present experiment were grown in media containing the same mineral nutrients and supplemented with the same carbohydrate, differences in growth behavior presumably reflect differences in responses to the vitamin supplements.

Results

Figure 2 shows the changes in volume of differently supplemented line 151 tomato root cultures measured at weekly intervals over a period of four weeks. In each instance average growth and growth of the best roots have been represented. The graphs have been prepared by combining the results from three sets of data obtained as outlined above.

At the end of one week in treatment one, the average volume of the roots was 0.020 ml. in a culture medium supplemented with thiamin, niacin, and pyridoxine. In no other culture solution did the roots nearly approach this volume after one week. It should be noted, however, that part of this advantage is undoubtedly derived from the fact that these roots were cultured in a completely supplemented medium during the so-called "weaning" period.

Roots in treatment two without any vitamin supplements were the smallest of the roots at the beginning of the experiment because they did not make any appreciable growth during the weaning period. The average increase over the four-week period was from 0.003 ml. to 0.017 ml. At the end of four weeks, the roots in treatment two had not reached the average size of the roots in treatment one at the end of one week.

Roots in treatment three in which thiamin only was added to the culture medium behaved essentially like those in the vitamin-free nutrient. This fact suggests that in this instance thiamin alone has no beneficial effect upon the roots.

To make certain that the factor involved here was not too low a concentration of thiamin (or any of the other vitamins) in the medium, an experimental series was run in which the concentration of each of the vitamin supplements was increased tenfold, that is, to one mg. per liter of thia-

min, five mg. per liter of niacin, and one mg. per liter of pyridoxine. The results from this series essentially duplicated those in the regular series. In no instance were there significant increases in growth; and in one or two instances there were suggestions that the higher concentrations were less effective, or perhaps slightly toxic.

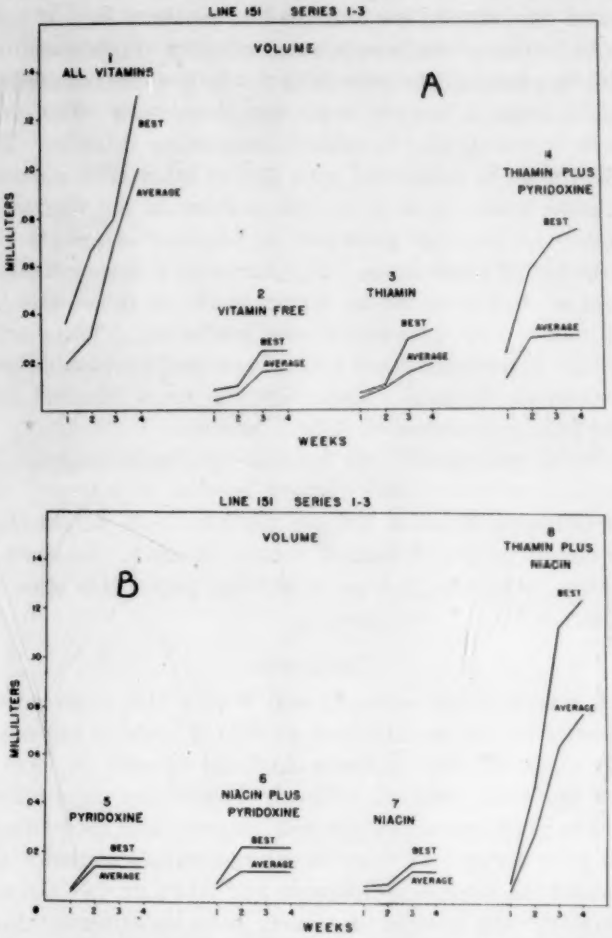

FIG. 2. A. Average volume changes in roots grown in the variously supplemented cultures. The changes in volume were, of course, by no means as abrupt as are indicated by these graphs which have been based on measurements at weekly intervals. The weekly interval, in some instances at least, obviously covers periods in which growth rate has changed markedly, but the time of this change is not detectable when measuremnts are spaced at this interval. B. Continuation of A.

Roots grown in treatment four which contained thiamin and pyridoxine showed a greater spread than those of most other treatments in the difference between average root growth and the growth of the best roots, but in

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both cases growth was distinctly better than in either the vitamin-free medium or the medium supplemented with only thiamin, and very significantly less than in the medium supplemented with all three vitamins.

Growth in treatments five, six and seven supplemented, respectively, with pyridoxine alone, niacin and pyridoxine, and niacin alone was uniformly poor. In no case was there any suggestion that growth in these solutions was any better than that in the vitamin-free medium.

Growth in treatment eight supplemented with thiamin and niacin gave results which approached the growth in the fully supplemented medium.

The results seem to suggest some complementary effect derived from adding niacin or pyridoxine to cultures containing thiamin. This complementary effect must be concerned with factors other than a simple increase in concentration inasmuch as a tenfold increase in the vitamin concentrations separately or together produced no increase in growth beyond that observed with unit concentration. Furthermore, it is specifically concerned with thiamin as supplemented by either niacin or pyridoxine. The combination of niacin and pyridoxine was ineffective. The complementary effect is further heightened when both niacin and pyridoxine are added to cultures containing thiamin. More vigorous roots resulted in every instance in the fully supplemented culture medium.

As was to be anticipated, the behavior of fresh weight (not shown) follows approximately the same pattern as that of volume. Dry weight changes are shown in figure 3. These, like the fresh weight changes, conform fairly closely to the pattern of volume changes. In some other lines of tomato roots to be reported on in another paper this close conformity does not exist.

Discussion

The early reports of ROBBINS (**8**) and WHITE (**19**) referred to yeast extract as essential for the satisfactory growth of roots in culture. In 1937 BONNER (**2**) suggested that thiamin chloride, vitamin B_1, was the active principle of the yeast extract. Bonner substituted crystalline thiamin chloride for the yeast extract in pea root cultures and found that the roots grew as well as or better than those in cultures supplemented with yeast extract. At about the same time ROBBINS and BARTLEY (**9**) showed thiamin to be essential for the growth of tomato roots in culture. Robbins and Bartley reported, however, that when thiamin was substituted for yeast extract in their tomato root cultures, growth was not quite as good as in cultures supplemented with yeast extract. On the basis of this fact, they suggested that thiamin would not act as a complete substitute for the yeast extract.

In 1938 ADDICOTT and BONNER (**1**) reported that growth of pea roots in thiamin-supplemented cultures was further enhanced by the addition of niacin. In 1939 ROBBINS and SCHMIDT (**11**) showed pyridoxine, vitamin B_6, to be a beneficial factor in tomato root growth. In the same year ROB-

BINS and SCHMIDT (**12**) evaluated growth of tomato roots in culture solutions containing thiamin, pyridoxine, and nicotinic acid. Both thiamin and pyridoxine had beneficial effects; the effect of pyridoxine being much greater when it was used in the presence of thiamin.

BONNER and DEVIRIAN (**3**) showed that roots isolated from the "beefsteak" variety of tomato require both thiamin and pyridoxine for satisfactory growth, and that growth could be further increased by the addition of niacin to cultures containing these two supplements. They reported that neither thiamin, pyridoxine, nor niacin alone would support appreciable growth.

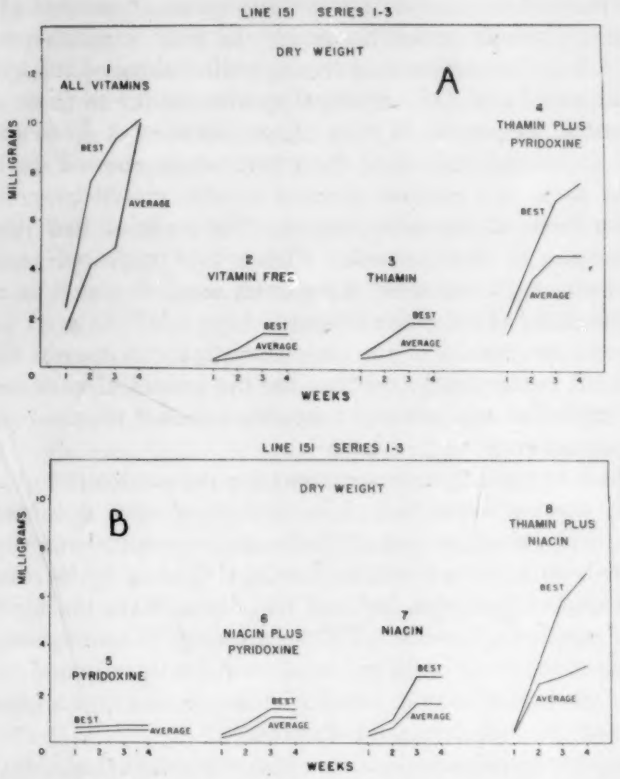

FIG. 3. A. Average dry weight changes in roots grown in variously supplemented media; measurements made at weekly intervals. B. Continuation of A.

In the following year WHITE (**21**) tested both his and Robbins' strains of tomato roots for responses to thiamin and pyridoxine. He concluded on the basis of his data that the beneficial effect of pyridoxine resulted from its capacity to act as a substitute for thiamin. In the same year BONNER (**4**) concluded that in five strains of tomato roots pyridoxine was an essential requirement, even though thiamin was present in the culture medium. His results indicate that all of his lines required thiamin, pyridoxine, and

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niacin, although all of them would make some growth without niacin. Bonner tested glycine and found that it could not replace pyridoxine in the nutrition of his root lines. In 1941 ROBBINS (**13**) analyzed the growth responses of roots of Johannesfeuer, a variety of *L. esculentum*, those of Red Currant, *L. pimpinellifolium*, and those of the hybrid between the two. Roots of Johannesfeuer showed greatest response to the addition of pyridoxine when thiamin was also present, less response to the addition of nicotinamide (the amide is apparently as active as niacin) in the presence of both thiamin and pyridoxine. The Red Currant roots made a greater response to nicotinamide than to pyridoxine. Roots of the hybrid grew more than those of the inbred lines in solutions supplemented with thiamin, pyridoxine and niacin or nicotinamide. In fully supplemented solutions the roots of Red Current grew nearly as well as those of the hybrid.

WHALEY and LONG (**15**) reported results similar to those of Robbins. They evaluated the growth of roots of two varieties of *L. esculentum*, Red River and Pritchard, and their F_1 hybrid which showed distinct hybrid vigor. The roots of Pritchard made a greater growth response to pyridoxine than those of the other inbred. The roots of Red River made a greater response to nicotinamide. The hybrid appeared to respond to both substances, but made about three times as much growth in a fully supplemented medium as did either inbred.

In general, the results of the early investigations suggest that thiamin must be added to the culture medium for the growth of most tomato roots, and that pyridoxine and niacin or nicotinamide are required by some but not all strains of roots.

Thiamin is apparently a requirement for the metabolism of all types of cells. It is supposed that one of its important roles is in carbohydrate metabolism. Inasmuch as most of the tomato root cultures on which growth studies have been made have reacted to added thiamin by increased growth, it can be assumed that such isolated root tissue lacks the ability to synthesize this essential substance. This seems a logical assumption in view of the evidence of the apparently universal need for thiamin and the fact that some forms can synthesize it provided thiazole and pyrimidine, the two components of the molecule, are both present.

The need for pyridoxine has been less well established. It appears to be important in the growth of many bacteria, yeasts and certain other fungi, but its rôle in the cellular metabolism of higher organisms has not been explored in any detail. A summary of the evidence for the utilization of pyridoxine by tomato roots seems to indicate a definite requirement of pyridoxine by certain lines if they are to make maximum growth. However, the lines which require it for maximum growth are usually capable of making some growth without it.

Niacin appears to be an essential or at least an important factor in the growth of several lower organisms. In most instances either niacin or

nicotinamide is effective, but in a few cases only nicotinamide has proved to be effective. Niacin is known to be important in the composition of coenzyme I and coenzyme II.

The results of the experiments reported here on tomato line 151 agree with the previously reported work in that greatest growth of the roots was found in cultures supplemented with thiamin, niacin, and pyridoxine. Growth in cultures which were not supplemented with any of the vitamins was very poor. The behavior of this line of roots differed from that of roots used in the earlier experiments particularly with respect to thiamin. In no instance did roots grown in a culture medium supplemented only with thiamin show growth appreciably greater than those grown in a vitamin-free medium. Taken alone, this fact might indicate either that thiamin is not essential for the growth of the roots of this particular line, or that these roots have an ability to synthesize sufficient thiamin for their own use. That neither of these assumptions is a part of the proper interpretation is made clear by the behavior of 151 roots in the cultures containing niacin alone or thiamin and niacin. Niacin alone does not raise the level of growth over that in the non-vitamin supplemented cultures, but niacin added to cultures which also contain thiamin produces roots which grow practically as much as those in the full vitamin supplemented cultures. When these facts are considered alongside the behavior of the roots in cultures supplemented only by pyridoxine or by thiamin and pyridoxine, a definite pattern of behavior for roots of this line appears.

It can be assumed from the data that thiamin is essential for the growth of line 151 roots, and that it is not synthesized by the roots themselves in sufficient amounts to produce good root growth. The absence of thiamin is a basic deficiency. However, the presence of thiamin does not produce satisfactory growth unless either pyridoxine or niacin or both are present in addition to the thiamin. Inasmuch as added pyridoxine produces much less response than added niacin, its lack can be considered either as a less important deficiency, or the assumption can be made that the roots have a capacity to produce some pyridoxine by their own metabolic processes. Lack of niacin is obviously an important deficiency, but the addition of niacin is effective only if the thiamin has also been provided. One can therefore postulate that roots of line 151 require thiamin for satisfactory growth and lack the ability to synthesize niacin from the materials available in the growth media. They may have some ability to synthesize pyridoxine, perhaps in less than optimum amounts. Complementary effects of these vitamin substances thus permit roots of line 151 to be classified as thiamin and niacin deficient, and probably weak in the production of pyridoxine for their own use.

Summary

Methods were evolved for determining volume changes of excised roots grown in culture. Volume increases of roots of University of Texas labora-

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tory line 151 of tomato were measured at seven day intervals and the data correlated with fresh and dry weight measurements of the same roots.

Line 151 roots grew best in White's solution with sucrose and glycine, supplemented with thiamin, niacin, and pyridoxine. They grew least in the absence of all of these vitamins.

The data indicate that line 151 roots require the addition of thiamin and niacin or pyridoxine for growth, and suggest complementary action of thiamin and either niacin or pyridoxine.

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THE GROWTH AND METABOLISM OF EXCISED ROOTS IN CULTURE. II. THE RESPIRATORY RATES OF EXCISED TOMATO ROOTS

GLENN S. RABIDEAU AND W. GORDON WHALEY

(WITH ONE FIGURE)

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Early studies on culture methods for excised roots have been summarized in review papers by WHITE (**8, 11**) and the adaptation of these methods to the experiments described here has been set forth in the first paper in this series (WHALEY, *et al.*, **7**). The method consists of growing roots aseptically in a solution containing inorganic salts supplemented with sucrose, with glycine, and with such vitamins or other accessory growth substances as are under investigation in a particular experiment.

The present series of experiments has been concerned with the roles of thiamin, niacin, and pyridoxine in the growth of roots of a particular line of tomato, *Lycopersicon esculentum* Mill, University of Texas Laboratory line 151. The growth responses of this tomato line have been discussed in detail by WHALEY, *et al.*, (**7**). The present paper is concerned with the correlations between these growth responses and respiratory rates, and thus with the possible influence of these vitamins on respiration.

The concept that the response of tomato root tissue to various organic supplements might be associated with respiration was suggested by WHITE (**9**). An earlier investigation of the oxygen relations of intact tomato roots had been made by CLARK and SHIVE (**1**). These investigators noted that an increased oxygen supply increased the growth of intact roots. The respiration of carrot and willow tissues grown in culture has been studied by PLANTEFOL and GAUTHERET (**6**). The influence of heteroauxin on the respiration of Jerusalem artichoke tissues grown in culture has been studied by LA CHAUX (**4**). HENDERSON and STAUFFER (**3**) have studied the effect of various respiratory inhibitors on the rate of respiration of isolated root tips of the "Bonny Best" variety of tomato by means of the Warburg manometric technique. They concluded that the respiration of such tomato roots is similar to that of other tissues in range of intensity, that minced root tissues or root slices did not have markedly higher respiration rates, and that changes in the oxygen content in the air above these tissues did not greatly alter the respiration rate.

The Warburg apparatus was also used by WHITE (**10**) to study the respiration rates of cultured crown-gall tissues. The results which White obtained indicated that such tissues did not differ greatly from normal intact tissues in their respiratory rates.

The relationship between the various vitamin requirements of genetic lines of tomato roots grown in culture and the respiration rates of these root

tissues has not been investigated. This problem is of fundamental importance in determining the nature of the metabolic processes involving vitamin deficiencies in various genetic lines of tomato. The purpose of the present investigation was to ascertain what differences, if any, exist in the respiration rates of isolated tomato root tissues when grown in White's medium variously supplemented with thiamin, niacin, and pyridoxine, both singly and in combinations.

Materials and methods

Excised roots of tomato line 151 were grown in White's solution containing 2% sucrose, glycine, and various vitamin supplements as follows: (1) complete (thiamin plus niacin plus pyridoxine); (2) vitamin free; (3) thiamin; (4) thiamin plus pyridoxine; (5) pyridoxine; (6) niacin plus pyridoxine; (7) niacin; and (8) thiamin plus niacin.

At weekly intervals two or three flasks of each vitamin treatment were selected for respiration measurements. The dry weight of the roots selected ranged approximately from one to 80 milligrams depending upon the growth which had occurred during the experimental period. Roots from cultures with identical supplements were combined and placed in the main well of a Warburg respirometer vessel. A separate respirometer vessel was used for each vitamin supplement. The roots were covered with a composite solution made from the solutions in which they had been grown. The vessel side arm contained 0.2 ml. of 1.25% NaOH to absorb the carbon dioxide released by respiration. The vessels were attached to Warburg manometers and shaken at the rate of 150 oscillations per minute in a water bath maintained within 0.05° C of the temperature of the culture room in which the roots had been grown (28° C). The vessels and their contents were allowed to equilibrate for a period of twenty minutes, after which the stopcocks were closed and readings of the oxygen uptake were taken at five minute intervals for a period of 70 minutes.

The root pieces were then removed from the vessels and the volume of the tissue from each Warburg vessel was determind by means of water displacement in a volumeter (WHALEY, *et al.*, **7**). After volume measurement the roots were transferred to tared weighing bottles and dried to constant weight at 105° C. Calculations of oxygen uptake on the basis of volume and of dry weight were made after application of the usual manometric constants. To provide further data for interpretation of the results in later experiments, and because of their possible significance in relation to respiration figures, acidity determinations were made with a pH meter on the original solutions and on the composite solutions in which the actual respiratory measurements were made.

Results and discussion

Table I shows the results of three series of measurements. The results are expressed as oxygen consumed per hour on the basis of unit volume of

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TABLE I

THE RESPIRATION RATES OF ISOLATED ROOTS OF TOMATO LINE 151 CULTURED IN WHITE'S MEDIUM WITH SUCROSE, GLYCINE, AND VARIOUS VITAMIN SUPPLEMENTS AS INDICATED. RESPIRATION WAS DETERMINED BY THE WARBURG MANOMETRIC TECHNIQUE. DASH LINES INDICATE AN INSIGNIFICANT AMOUNT OF RESPIRATION.

VITAMIN SUPPLEMENT	MM^3 OXYGEN/ML. VOLUME OF TISSUE/HR.				MM^3 OXYGEN/CG. DRY WEIGHT OF TISSUE/HR.			
	AGE OF CULTURE IN WEEKS				AGE OF CULTURE IN WEEKS			
	1	2	3	4	1	2	3	4
			1ST SERIES					
1. All Vitamins	590	280	360	170	43	36	23	17
2. Vitamin Free	260	—	—	—	—	—	—	—
3. Thiamin	390	260	—	—	—	7	—	—
4. Thiamin, Pyridoxine	520	390	250	100	37	35	21	3
5. Pyridoxine	260	—	—	—	—	—	—	—
8. Thiamin, Niacin	—	590	390	350	—	69	38	14
			2ND SERIES					
1. All Vitamins	795	765	310	65	63	22	20.5	4
4. Thiamin, Pyridoxine	960	670	570	190	83	34	32.5	10
8. Thiamin, Niacin	—	995	510	145	—	51	13	11
			3RD SERIES					
1. All Vitamins	1070	550	720	410	61	64	42	28
4. Thiamin, Pyridoxine	600	1200	440	480	112	112	35	34
8. Thiamin, Niacin	800	—	990	—	90	—	72	—

root and unit dry weight of root. In general, the respiration rate on the dry weight basis falls within the range for tomato root tissue found by HENDERSON and STAUFFER (3). It was established in preliminary experiments (only a few of which are summarized in table I) that there is a measurable, but low, respiration rate in vitamin-free cultures, at least for the early sampling periods. These cultures were observed to grow but very little. When either niacin or pyridoxine is added to cultures containing thiamin, root growth is very much better than in the vitamin-free medium or single vitamin-supplemented media. Cultures containing thiamin and niacin or pyridoxine also exhibit striking increases in the rate of respiration over those lacking vitamins or having only a single vitamin supplement. That the effect is a complementary one involving thiamin as the basic deficiency is seen from the fact that thiamin supplemented with either niacin or pyridoxine supports growth very effectively, but that niacin and pyridoxine in the absence of thiamin do not support growth at an appreciably higher rate than in the vitamin-free medium. (WHALEY, *et al.*, 7.) This was also found to be true with regard to root respiration. A similar complementary effect of thiamin and pyridoxine has been observed independently by LEE (Unpublished, 5) working with isolated roots of a line of tomato derived from the variety Pritchard. In the earlier paper in this series (WHALEY, *et al.*, 7) it was noted that there seemed to be a "double" or heightened complementing effect when both niacin and pyridoxine were

added to cultures containing thiamin. This growth difference does not seem to be reflected in higher respiration rates, but it is, of course, not possible, within the experimental limits of the techniques used, to state that at certain times the cultures containing all three vitamins may not have had higher respiration rates. It is possible to state only that when measured, the respiration rates of the full vitamin-supplemented roots were not uniformly higher than those grown in cultures containing thiamin and niacin or thiamin and pyridoxine.

Respiration data from the experiments with line 151 are summarized in graphic form in figure 1. It is apparent from inspection of either table I or figure 1 that in all cultures there was a decrease in the rate of respira-

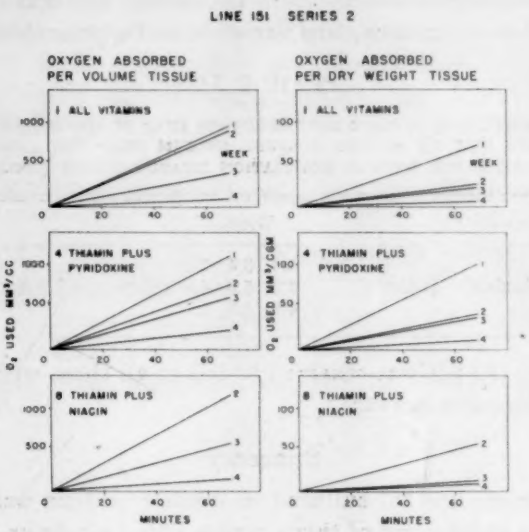

FIG. 1. Respiration rates of isolated roots of tomato line 151 showing weekly changes in the rates.

tion with increase in age of the root tissue. This is true for respiration rate calculated on either the volume or the dry weight basis. The growth data reported in the earlier paper (WHALEY, *et al.*, **7**) show that in the fully supplemented culture medium there is a progressive root size increase for at least four weeks. These total size increases with time are not reflected in increased respiration rates, but are accompanied by decreasing respiration rates. Had the total weights and volumes in the earlier paper been plotted on a weekly increment basis as has been done by FIEDLER (**2**) they would not have shown continuously larger values with time but rather decreasing values for many of the measurements after the first week. The respiration decreases are real rather than apparent decreases which have arisen because of the progressive increase in volume and dry weight of tissue during growth. This can be shown by comparing the ratio of the maximum and minimum

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respiration rates with the ratio of the corresponding minimum and maximum dry weights. In almost all cases the ratio of the decrease in the rate of respiration exceeds the ratio of the increase in dry weight, in some cases by as much as two to four times. This means that the decrease in the rate of respiration cannot all be accounted for by the synthesis of cellulose walls and other non-respiring cellular and extra-cellular materials. But whether this respiration decrease is the result of the accumulation of substances toxic to growth and metabolism, to an exhaustion of the supply of some of the essential growth substances, or to other factors, could not be determined on the basis of the present experiments. Data bearing on this question will be discussed in a later paper.

Results of acidity measurements of the culture media are shown in table II. Although some cultures show variation and a general increase in alkalinity with age, the pH was observed to fall at all times within the physiological range for plant growth.

TABLE II

ACIDITY MEASUREMENTS OF TOMATO ROOT CULTURES USED IN RESPIRATION STUDIES. EACH FIGURE REPRESENTS THE pH OF THE CULTURE MEDIUM FROM THE INDIVIDUAL CULTURES COMPOSITED FOR WEEKLY RESPIRATION MEASUREMENTS (SERIES 2).

VITAMIN SUPPLEMENT	WEEK 1	WEEK 2
1. All Vitamins	5.4, 5.4, 5.2	5.1, 5.8, 5.7
4. Thiamin Pyridoxine	4.9, 5.0, 4.8	5.0, 4.5, 5.4
8. Thiamin Niacin	4.6, 4.5, 4.7	4.4, 4.5, 4.5

Summary

Roots of tomato line 151 cultured in White's medium were found to require a vitamin supplement of thiamin plus either niacin or pyridoxine for satisfactory growth. In cultures which showed visible growth, the respiration rate, as determined by the Warburg manometric method was significantly higher than that of cultures in which there was no visible growth.

Decrease in the respiration rate accompanied aging of the cultures.

The pH measurements of the individual cultures indicated a satisfactory range for plant growth.

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A SOFT X-RAY MUTANT OF *ASPERGILLUS NIGER* VAN TIEGHEM

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(WITH FIVE FIGURES)

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Introduction

A culture of *Aspergillus niger* van Tieghem (American Type Culture Collection No. 1004) was subjected to soft X-rays. From this treatment there was obtained a mutant strain differing in appearance and metabolism from the parent culture. The mutant and normal strains were investigated with respect to their individual growth characteristics, glucose utilization, citric acid production, and response to growth factors; and chemical analyses were made of the dried mats.

Production of the mutant

A Petri dish colony of *A. niger* was subjected to a beam of soft X-rays from a gas X-ray tube (**9**) for producing X-rays ranging from about 0.5 Angstroms to 4 Angstroms. This tube is equipped with a copper target and a window of cellophane and aluminum which transmits the K_α and K_β lines of copper (around 1.5 Angstroms) as well as the longer and shorter wavelengths in the continuous spectrum. The irradiation data were:

Age of culture at time of irradiation	90 hours
Distance from window of X-ray tube	5 centimeters
Tube voltage	30 kilovolts
Tube current	10 milliamperes
Time of exposure	15 minutes

It is possible to arrive at an evaluation of the dosage employed by referring to the curves given by KREBS and KERSTEN (**10**) in their calibration of this soft X-ray tube. Operation at 30 peak kilovolts and 10 milliamperes produces a dosage of 1650 roentgens per minute at a distance of 5 centimeters from the window.

Isolation of the mutant

After the irradiation, the culture was scraped with a loop and serial dilutions made onto Sabouraud's maltose agar plates, which were then incubated at 37° C for seven days. The plates were inspected for isolated colonies which differed in gross appearance from the normal *A. niger* culture. This difference was manifested by a reduction of pigment formation; colonies were found which were light brown, tan, and cream-colored in addi-

tion to a larger number which were the usual dark brown of *A. niger*. The atypical colonies were transferred to slants and one was chosen at random for detailed analysis. In order to insure a pure culture, the mutant culture was subjected to a plating-out method.

General characteristics of the normal and mutant strains

The normal culture has the typical appearance of *Aspergillus niger* van Tieghem (fig. 1) as described by THOM and RAPER (**23**). Colony growth is rapid and conidia are brownish-black to black in color.

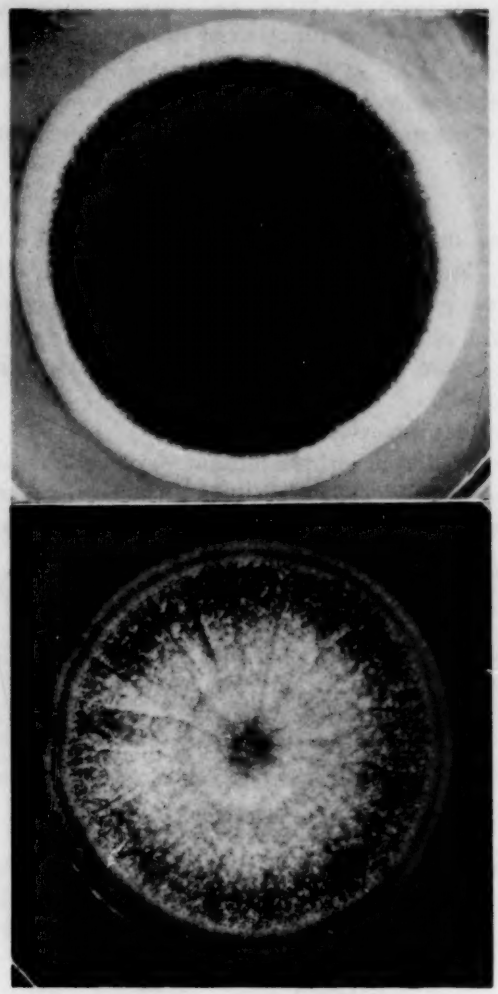

FIG. 1. Colonies on Sabouraud's maltose agar medium; single spore inoculation; photographed at nine days. Upper: *A. niger*. Lower: Mutant.

The mutant (fig. 1) differs in several respects from the normal strain. The sporulation is definitely reduced, to such an extent that there are patches

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of mycelial growth which show very few spores. The conidia are very light cream to tan in color. Viewed from the underside, a slant or plate culture is yellow-tan in color and does not darken with age. This is in contrast to the appearance of a normal *A. niger* culture, the underside of which becomes darker as the culture ages and the dark brown pigment diffuses through the agar medium.

Under the microscope, typical Aspergillus conidial heads are seen in both the normal and mutant strains.

Growth, glucose utilization, and citric acid production as a function of time

Since citric acid is one of the major metabolic products of *A. niger*, the normal and mutant strains were compared in this respect; at the same time the citric acid synthesis was correlated with mat production and glucose utilization.

Culture medium and method

The liquid medium used was a modification of Czapek's medium and contained 2.4 gm. $NaNO_3$, 0.6 gm. NH_4NO_3, 1.0 gm. KH_2PO_4, 0.5 gm. $MgSO_4 \cdot 7H_2O$, 0.5 gm. KCl, 10 mg. $FeSO_4 \cdot 7H_2O$, 2.0 gm. Difco yeast extract, and 100 gm. anhydrous dextrose per liter. The pH was adjusted to 3.6 with HCl. After autoclaving, 50 ml. of this medium were pipetted aseptically into each of 100 wide-mouth (8 oz.) bottles, which had been plugged and sterilized previously.

Single spore inoculations of the mutant were made in half of the bottles and of the normal strain in the other half. The bottles were incubated in a slanting position (fig. 2). Beginning with the second day of incubation,

Fig. 2. Growth on liquid medium at eight days. Left: *A. niger*. Right: Mutant.

five bottles of each of the two strains were removed daily for determination of dried mat weight and chemical analyses for glucose and citric acid in the medium. Analyses were made through eight days of growth.

Chemical methods

Dried mat weight was determined by filtration through tared Gooch crucibles prepared with asbestos mats. The mold mats were dried at 100° C for 24 hours. The analysis for glucose was done by the Shaffer-Hartman (**20**) modification of the Munson-Walker (**15**) method. Citric acid

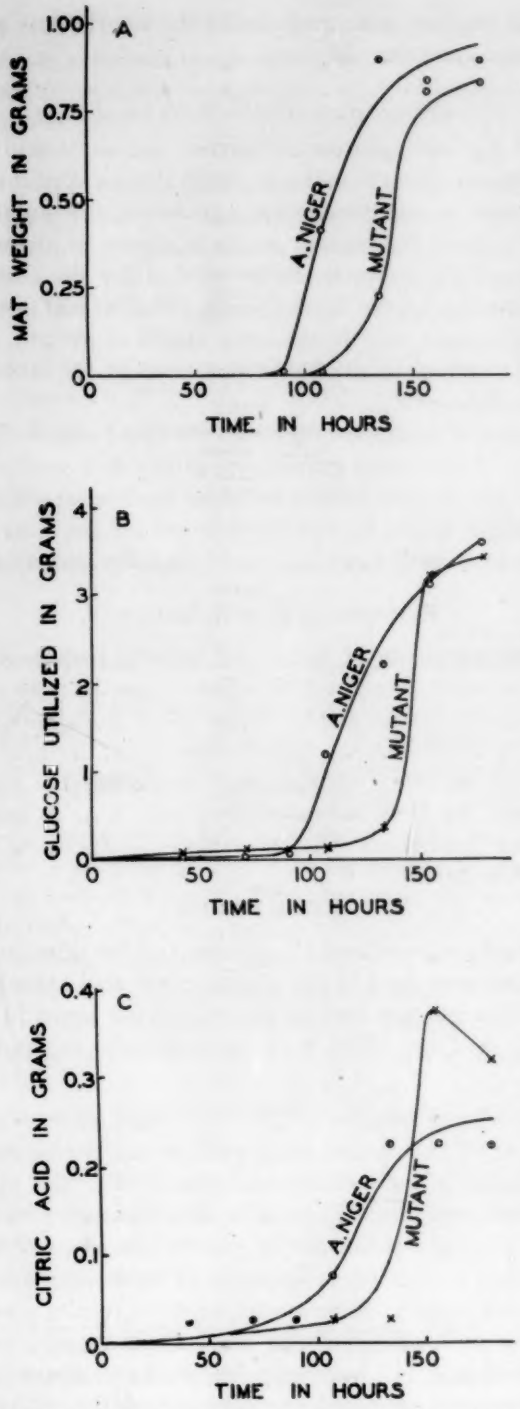

FIG. 3. Mat weight, glucose utilization, and citric acid production as a function of time, *A. niger* and mutant.

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analyses of the medium were made using the colorimetric method of PERLMAN, LARDY, and JOHNSON (16).

RESULTS

The data for growth, glucose utilization, and citric acid production as a function of time are given in figure 3. Both strains exhibit a normal growth curve with respect to mat production. However, the mutant curve lags at all times. In general the mutant strain is slower to utilize glucose in the medium, although the curve is similar to that for the normal mold. The citric acid production by the normal strain parallels mat production but this is true for the mutant only in the early stages of growth, since there is a sudden 1800% increase in citric acid produced by the mutant between 135 and 156 hours of growth.

The difference in metabolism between the two forms is illustrated by the following data. The mutant grows more slowly and produces less mat; the ratio of citric acid to mat weight is larger for the irradiated culture; the ratio of mat weight to glucose utilized is larger for the mutant; and the mutant strain produces 50% more citric acid than the normal *A. niger*.

Response to growth factors

Various concentrations of individual growth factors were added to a basal medium in order to compare the effects upon the growth of the mutant with those upon the normal mold. Single spore inoculations were made on solid medium in Petri dishes. Growth was determined by measuring the diameters of the resulting colonies and calculating the areas. For each growth substance, the areas were compared with those on basal medium, the two values being determined simultaneously. The cultures were incubated at 37° C for seven days.

BASAL MEDIUM

The basal medium contained 1% glucose and the same concentrations of inorganic salts as were used in the glucose-citric acid experiment described previously. Silica gel was used as the solidifying agent in order to avoid the use of agar and any effect it or its impurities might have had upon growth (18).

RESULTS

A comparison of the normal mold and the mutant in respect to nutritional requirements is summarized in table I. For the purposes of this table, the greatest per cent. of increase in area obtained with each substance was calculated. It was found that the growth factors could be divided into groups, each group producing percentages of increase in growth which fell into well separated ranges. Representative of the results are the pictures of the increase in growth obtained with three of the substances, para-aminobenzoic acid, riboflavin, and hypoxanthine, shown in figure 4.

Difco yeast extract produced the most stimulation in both strains, al-

TABLE I

COMPARISON OF NUTRITIONAL REQUIREMENTS OF *A. niger* AND MUTANT

AVERAGE PER CENT. OF INCREASE IN GROWTH		
A. niger		Mutant
> 190%	yeast extract	> 460%
70%	biotin	240%
10–50%	p-aminobenzoic acid hypoxanthine inositol pyridoxamine	140–200%
< 20%	casein hydrolysate cytosine monohydrate guanine hydrochloride niacin pyridoxine hydrochloride pimelic acid riboflavin thymine	40–75%
< 20%	adenine sulphate choline chloride thiamin hydrochloride uracil	< 20%
50%	uric acid xanthine	< 20%

though the increase in growth over that on basal medium was much greater for the mutant than for the normal strain. Of the individual substances, biotin was outstanding in producing 240% increase in growth in the mutant in contrast to 70% in the normal mold. Substances in the group which included para-aminobenzoic acid, hypoxanthine, inositol, and pyridoxamine produced 140 to 200% increase in growth in the mutant in contrast to 10 to 50% increase in the normal *A. niger*. The next group containing "vitamin-free" casein hydrolysate, cytosine, guanine, niacin, pyridoxine, pimelic acid, riboflavin, and thymine, stimulated both strains to a lesser degree, there being 40 to 75% increase in the mutant and less than 20% in the control mold. Adenine, choline, thiamin, and uracil produced an insignificant amount of stimulation in the two strains. Uric acid and xanthine were the only substances tested which stimulated the normal *A. niger* more than the mutant, 50% increase in growth having been found for the normal strain in contrast to less than 20% for the mutant.

In view of the above mentioned data obtained with Difco yeast extract and growth factors, it can be concluded that the mutant strain is apparently unable to synthesize, at the rate of the normal strain, a number of substances important for growth.

Analyses of the nucleic acid fractions of the dried mycelium

A. niger and mutant hyphal mats, grown on liquid medium, were dried with ethanol and ether. The acid-soluble and the lipoid portions of the dried

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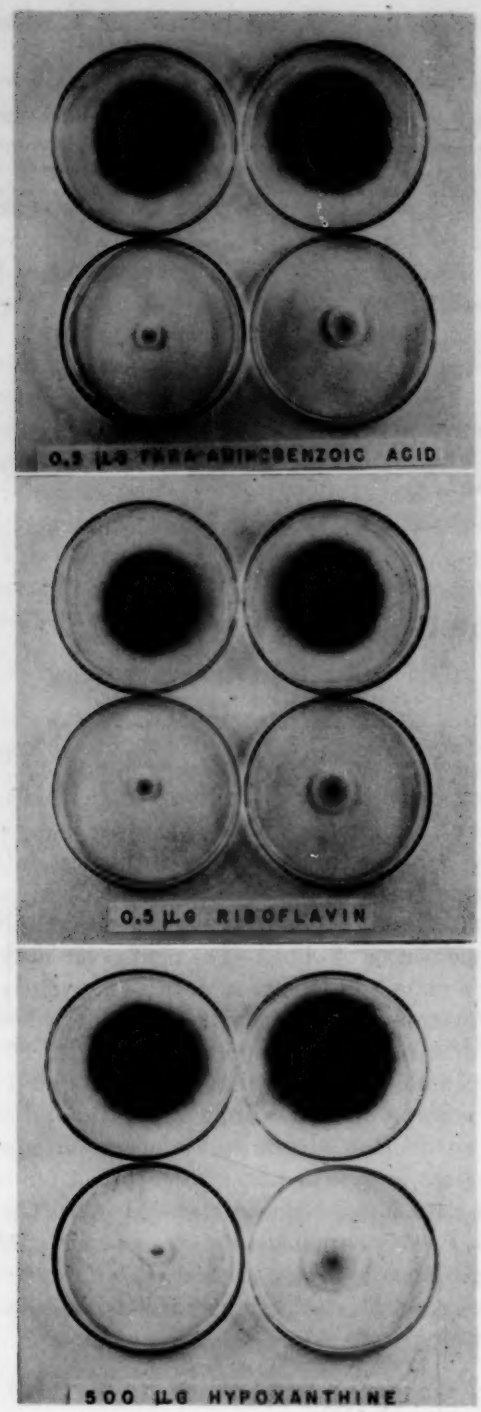

FIG. 4. Changes in area of colonies with the addition of para-aminobenzoic acid, riboflavin, and hypoxanthine to a basal medium. In each of the three photographs: *A. niger* on basal medium (upper left plate), on medium with growth factor (upper right plate); mutant on basal medium (lower left plate), on medium with growth factor (lower right plate).

mats were removed by extraction. The nucleic acid fraction was then extracted with 10% sodium chloride and precipitated from the sodium chloride solution by lanthanum. Determinations of nitrogen, phosphorus, and pentose (ribose and desoxyribose) were made on the lanthanum precipitable portions of the normal mold and the mutant. In addition the ultraviolet absorption characteristics of the nucleic acid fractions were investigated.

Preparation of dried mycelium

The medium used for the growth of the mycelium was the modified Czapek's medium previously described except that yeast extract was omitted. The two strains were grown in 500 ml. Erlenmeyer flasks each containing 100 ml. of the medium. After incubation at 37° C for seven days, the medium was decanted and discarded. The mold mats were washed several times with large portions of distilled water and then removed from the flasks to paper towelling which absorbed surplus moisture.

The mats were cut into small pieces, dried by washing with (1) ethanol, (2) ethanol-ether mixture, (3) ether, and then put into a vacuum desiccator. After several days, the material was ground in a mortar and then returned to the desiccator for the final drying.

Extraction procedure and lanthanum precipitation

The method of extraction was that of Davidson and Waymouth (**5**), except for several minor modifications necessitated by the fact that dried mold mycelium rather than animal tissue was being investigated. Three samples were extracted with hydrochloric acid, ethanol-chloroform, and sodium chloride according to this method. A fourth sample was extracted with trichloroacetic acid. This was suggested by the work of Mann (**12**) on the phosphorus content of *A. niger*. The trichloroacetic acid was more effective in removing metaphosphate which was present in the samples of normal mold mat. The extraction was done with six portions of trichloroacetic acid: (1) 10% at 0° for one hour, (2) four portions of 5% at 0° for one hour each, and (3) 5% at 0° overnight.

The lanthanum precipitable fractions (assumed to be nucleic acids) of the four sodium chloride extracts were obtained using lanthanum nitrate, and these fractions were used for the various analyses.

The extraction method of Davidson and Waymouth (**5**) was checked, using the procedure of Schneider (**19**). The latter method is based on the extraction of the nucleic acids with hot trichloroacetic acid and analysis of the resultant extract for the specific pentoses and purines. The two methods yielded approximately equivalent data.

Chemical analyses

Phosphorus determinations were made according to the method of Fiske and Subbarow (**6**). Nitrogen determinations were made by the colorimetric

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micro-method which employs Nessler's reagent (**7**). Calculations of nucleic acid content were made, assuming nucleic acid equals approximately six times the nucleic acid nitrogen concentration.

Ribose was determined by two methods: that employing the aniline acetate reagent (**17**) and the perchloric acid-tryptophan method (**4**). It should be remembered that with the first of these methods one can expect recovery of no more than 50% of the ribose content of the sample. Desoxyribose determinations were made with the method employing the diphenylamine reagent (**5**) and the perchloric acid-tryptophan method (**4**). Calculation of desoxyribosenucleic acid content was made by comparison with a standard curve prepared with various amounts of sodium desoxyribosenucleate.

Total purines

Analyses were made on the lanthanum precipitable fractions and the hot trichloroacetic acid extracts for total purines according to the method of Hitchings (**8**).

Ultraviolet absorption

Various ultraviolet absorption curves were obtained with a Beckman quartz spectrophotometer using the wavelength region from 235 to 300 millimicrons. The following data were collected:

(1) Variation in optical density with wavelength for yeast nucleic acid and sodium desoxyribosenucleate, (fig. 5). The ribosenucleic acid (Schwartz) which was used was purified by reprecipitation from acetic acid according to the method of Kunitz (**11**). The desoxyribosenucleic acid was prepared as the sodium salt from calf thymus by the methods of Mirsky and Pollister (**14**) and McCarty (**13**).

(2) Variation in optical density with wavelength for the lanthanum precipitable portions of the sodium chloride extracts obtained from four samples of normal *A. niger* and the mutant (fig. 5).

(3) Total nucleic acid concentrations of the four samples of *A. niger* and the mutant, based on a standard ribosenucleic acid curve obtained by getting variation in optical density for various concentrations of yeast ribosenucleic acid with wavelength fixed at 260 millimicrons (**3**).

Results

Figure 5 shows that the lanthanum precipitable portions of the sodium chloride extracts of the dried mats of *A. niger* and the mutant have ultraviolet absorption curves typical of nucleic acid, with maximum absorption at 2600 Angstroms.

An analysis of the nucleic acid fractions by the two methods for total phosphorus, total nitrogen, ultraviolet absorption, pentose, and total purines shows conclusively (table II) that the nucleic acid content of the normal mold is approximately 30% higher than in the mutant. The ultraviolet absorption curves indicate that the nucleic acid fraction is typical of nucleic

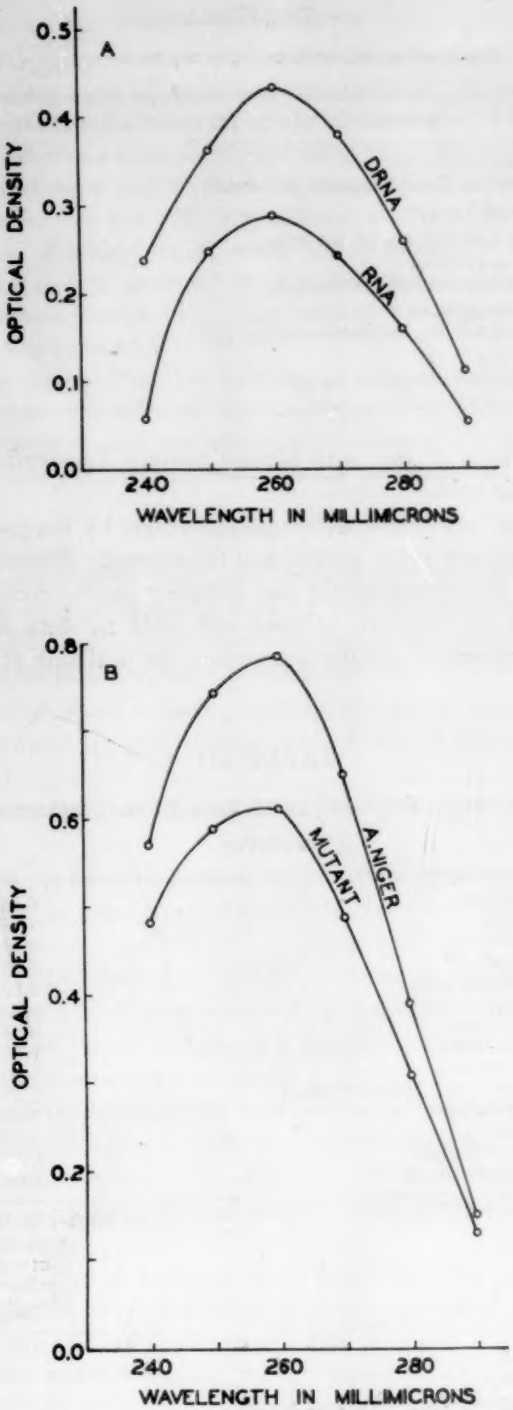

FIG. 5. A: Ultraviolet absorption curves for solutions of ribosenucleic acid prepared from yeast (12.5 μg. RNA per ml.) and sodium desoxyribosenucleate prepared from calf thymus (20 μg. DRNA per ml.). B: Ultraviolet absorption curves for the lanthanum precipitable portions of the sodium chloride extracts of *A. niger* and mutant.

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TABLE II

RATIO OF TOTAL NUCLEIC ACID FRACTIONS $\frac{\text{NORMAL}}{\text{MUTANT}}$

METHOD	RATIO N/M
Phosphorus of La ppt. (corrected for metaphosphate in normal)	1.33
Nitrogen of La ppt.	1.36
Absorption at 260 mμ of La ppt.	1.31
Ribose-desoxyribose (tryptophan-perchloric on La ppt.)	1.41
Ribose-desoxyribose (aniline acetate-diphenylamine on La ppt.)	1.27
Total purine (method of Hitchings on La ppt.)	1.43

acid and that both of the mold strains contain desoxyribosenucleic acid and ribosenucleic acid.

There was no qualitative difference detectable by the methods used between the nucleic acid of the normal and the mutant. There was a quantitative difference: the normal strain had a higher concentration. It must be emphasized that the methods utilized will yield no data as to molecular size, type of linkage, or details concerning the intimate structure of the nucleic acids.

TABLE III

RATIO $\frac{\text{NORMAL}}{\text{MUTANT}}$ OF AMINO ACIDS BASED ON PER CENT. TOTAL N CORRECTED FOR ASH AND MOISTURE

Arginine	0.70
Valine	1.37
Histidine	0.91
Tyrosine	1.95
Glutamic acid	0.25
Leucine	0.93
Isoleucine	0.84
Methionine	0.80
Threonine	1.00
Serine (and hydroxylysine)	1.10*
Tryptophan	1.05
Lysine	0.78†
Phenylalanine	0.73
Cystine	1.08

Higher in Normal	Higher in Mutant
Valine	Arginine
Tyrosine	Glutamic acid
	Lysine
	Phenylalanine

* Chemical assay.
† *S. faecalis* assay.
All other assays by *Cl. perfringens* method.

Amino acid analyses

STANLEY (**21**) reported that mutant strains of tobacco mosaic virus showed striking differences in amino acid composition. To investigate this point complete quantitative analyses of the amino acids of the mold mats were made. The analyses were performed using the microbiological method outlined by BOYD, LOGAN, and TYTELL (**2**), employing *Clostridium perfringens*. *Streptococcus faecalis* (**22**) was utilized also as a check method for the determination of lysine, which could not be assayed with *Clostridium perfringens*. Serine was analyzed by the periodic oxidation method described by BOYD and LOGAN (**1**). This method actually measures hydroxylysine also if it is present.

The analyses showed that the qualitative composition with respect to amino acids assayed was identical for the two strains. The mutant (table III) was significantly higher in arginine, glutamic acid, lysine, and phenylalanine. The normal mold was higher in valine and tyrosine.

Summary

A colony of *Aspergillus niger* van Tieghem was subjected to a beam of soft X-rays. An atypical strain of *A. niger* was isolated from the irradiated culture. Nutritional and biochemical studies on the normal and mutant strains can be summarized in the following manner:

1. Pigmentation in the mutant is practically absent; sporulation is very much depressed; and the growth rate is much slower than for the normal strain.

2. The mutant is more efficient in the production of citric acid from glucose.

3. The mutant is more responsive to individual growth substances, indicating a general reduction in rate of synthesis of these substances on the part of the mutant.

4. Amino acid analyses of the mold mats indicate that qualitatively the amino acid composition of the proteins of the mutant and normal are identical. Quantitatively the mutant shows a significant deviation from the normal with respect to several amino acids.

5. Analysis of the total nucleic acid content of the mold mats indicates that a significantly decreased amount of nucleic acid per unit mat weight is produced by the mutant. There is no distortion of the desoxyribosenucleic acid/ribosenucleic acid ratio in the mutant nucleic acid as compared with the normal strain.

6. When harvested at seven days, no metaphosphate was found in the mutant. The normal produced metaphosphate as reported by MANN (**12**).

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BRIEF PAPERS

A MODIFICATION OF THE WARBURG RESPIROMETER TO MEASURE THE RESPIRATION RATE OF TOMATO LEAF DISCS[1]

J. Edward Klinker

Received January 19, 1950

In connection with a physiological study of six tomato varieties, the need for a method of measuring simultaneously the respiration rate of the leaves of several replicates of these varieties arose.

Bonner and Wildman (**1**) have reported on the use of the Warburg apparatus for measuring the respiration of spinach leaves. They obtained highly reproducible rates of gas exchange from 200 milligram samples of fresh spinach leaf fragments 5–10 mm^2 in area, when mixed with two milliliters of a phosphate buffer and measured in the Warburg respirometer.

Preliminary attempts were made to measure respiration of tomato-leaf discs by the oxygen absorption method discussed by Umbreit, Burris and Stauffer (**3**). Twenty discs were cut from a tomato leaf with a one-quarter inch cork borer. Oxygen uptake measurements of these discs were made in a darkened room in Warburg flasks. Two-tenths milliliter of 20% potassium hydroxide was used in the center well of each flask to absorb the carbon dioxide. All measurements were made at 25° C, using an M/15 potassium-dihydrogen-phosphate buffer with a shaker rate of 120 strokes per minute. Extremely variable rates of oxygen uptake were obtained between replicates of discs taken from the same leaf.

It seemed reasonable to suspect that the buffer might be the principal cause of these erratic rates of oxygen absorption. The buffer being in close contact with the leaf discs, would greatly impede the normal exchange of gases in respiration. This interference would be especially great in the absorption of oxygen since its solubility in water is quite low. From Lange's Handbook of Chemistry (**2**) the α value for oxygen at 25° C is 0.0283; whereas the α value for carbon dioxide at 25° C is 0.759, or carbon dioxide is approximately 27 times more soluble in water than is oxygen.

Subsequently a modification of this method was tried in which the buffer was eliminated and filter paper of uniform size was cut and fitted in the bottom of the Warburg flasks. One-tenth milliliter of water was pipetted into the bottom of each flask to moisten the filter paper. Twenty tomato leaf discs were then placed in regular order on the filter paper. The flask atmosphere was kept sufficiently moistened by the wet filter paper to maintain turgor of the leaf discs. Two-tenths milliliter of 20% potassium hydroxide was used in the center well to absorb the carbon dioxide. With the Warburg apparatus available for this work, simultaneous respiration determinations could be made of leaf discs from two complete replicates of six varieties each plus a thermobarometer. All measurements were made at 28° C in a darkened room.

[1] Paper No. 331, Department of Vegetable Crops, Cornell University, Ithaca, New York.

Two runs of two replicates each were made, each run being one hour in duration. After measurements of oxygen uptake were completed, the disc samples were dried to constant weight and their weights recorded. Calculations were made to convert the oxygen uptake values to microliters uptake of oxygen per milligram dry weight of the leaf discs. A summary of these data is given in table I.

TABLE I

OXYGEN CONSUMPTION OF LEAF DISCS OF SIX TOMATO VARIETIES.
(MICROLITERS O_2/MG. DRY WT./HR)

VARIETY	REPLICATION				MEAN
	1	2	3	4	
46–31	2.26	1.62	2.31	2.21	2.10
Gem	3.02	2.25	2.22	2.92	2.60
John Baer	2.48	2.37	2.24	2.22	2.33
Longred	2.14	1.90	1.75	2.71	2.12
Rutgers	2.39	2.09	1.87	2.17	2.13
Improved Wasath Beauty	2.12	1.64	1.92	2.07	1.94

The data in table I show fairly uniform readings between replicates of the same variety. The statistical analysis revealed that the calculated F value for varieties exceeded the required F value at the .05 level of significance. Hence, the varieties, as measured by this method, had significantly different rates of leaf respiration. This would indicate that determinations made by this modified method were much more consistent than were those made when a buffer was used.

One obvious source of error in this method involves wounding. Discs cut from leaves probably do not carry on respiration at the same rate as intact leaves due to wounding. In the case of measuring the rate of respiration of one plant relative to another, as was required for this study, this error does not seem serious.

The author wishes to express his appreciation to Dr. G. Fred Somers and Dr. William C. Kelly of the U. S. Plant, Soil and Nutrition Laboratory, Ithaca, New York, for their many suggestions and aid in the development of this modified method.

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PRECAUTIONS IN THE USE OF LANOLIN AS AN ASSAY DILUENT FOR PLANT GROWTH SUBSTANCES[1]

C. T. Redemann, S. H. Wittwer, and H. M. Sell

(WITH ONE FIGURE)

Received November 30, 1949

Lanolin is frequently used as a diluent for applying growth substances to plants. Its earliest use was described by Laibach (**1**). He applied unilaterally, to the first internodes of *Phaseolus multiflorus* seedlings, a lanolin paste containing the test substance. The presence of growth substances induced negative stem curvatures. Subsequently, Laibach and co-workers (**2, 3**) applied lanolin pastes containing dissolved ether-extractible materials of urine and orchid pollen to the cut stems of Coleus and Tradescantia. Callus formation and rooting were induced. Numerous other workers have since adopted lanolin as a diluent. The use of lanolin has persisted largely because of its desirable physical characteristics, and its apparent inertness toward plant tissues and growth substances.

An adaptation of the bio-assay method described by Laibach (**1**) was adopted in studies designed for the eventual isolation of the growth regulating substances in corn pollen. The degree of enrichment obtained from any particular fractionation was measured by the curvature produced on bean seedlings. By successive dilutions, determinations were made of the lowest concentrations dissolved in lanolin, which would produce curvatures of the same magnitude as would the non-diluted starting material. In earlier isolation attempts, even under the best processes, a considerable loss of activity sometimes occurred. It was found that upon standing for 24 hours at room temperature in contact with a 1% aqueous solution of hydrogen peroxide much of the growth regulator present in the crude pollen extracts was destroyed. Presumably, other oxidizing agents might also react with the growth regulator(s). Removal of peroxides from all solvents employed in extraction and enrichment processes did not eliminate the loss of activity indicated by the lanolin dilution assays. The probable source of this loss was detected when a qualitative test with acidified potassium iodide showed that the sample of lanolin employed as a diluent contained unidentified oxidizing agents.

Subsequently, it was found that treatment of the lanolin with sodium hydrosulphite removed the oxidizing agents. A product satisfactory for bioassay of natural growth substances may be obtained by shaking out a solution of 25 gm. of lanolin in 250 ml. of ether with 100 ml. of water in which are dissolved 15 gm. of sodium hydrosulphite and 5 ml. of glacial acetic acid. After separating the two phases, the less dense phase (ether)

[1] Journal Article No. 1105 (n.s.) of the Michigan Agricultural Experiment Station.

is washed with 100 ml. of water. The phases are again separated, and residual water is removed from the ether solution by agitating it with 15 gm. of anhydrous sodium sulphate, and the ether solution is evaporated to dryness over a steam bath. Fifteen ml. of water is then added and subsequently evaporated to dryness over a steam bath *in vacuo*. The residue from this second evaporation makes a satisfactory assay diluent.

Differences in the curvatures of bean seedlings which were observed when lanolin treated with sodium hydrosulphite was substituted for U.S.P. anhydrous lanolin are illustrated in Figure 1. Solutions containing 0.01, 0.001,

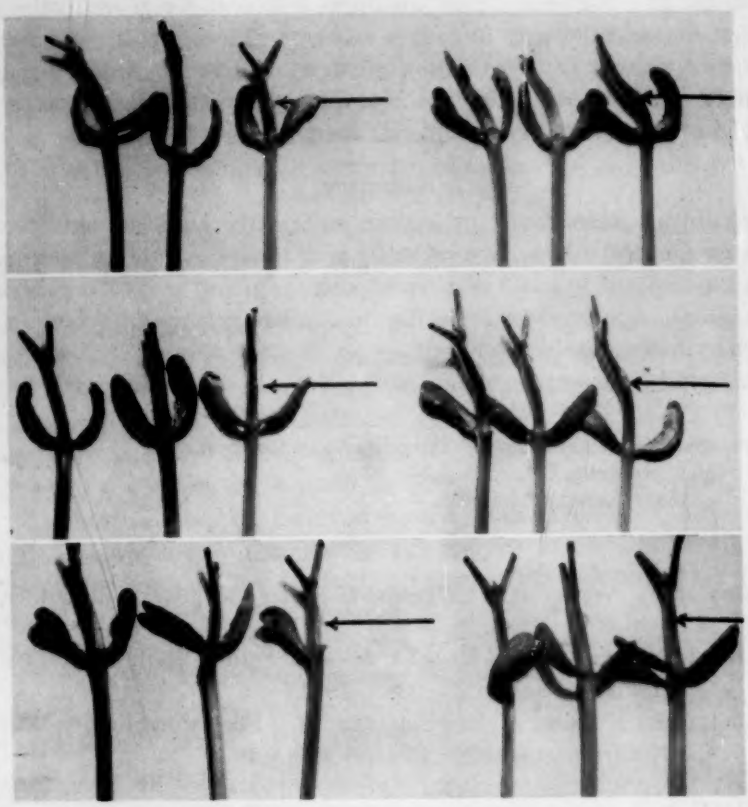

FIG. 1. Curvatures induced on bean seedling internodes six hours after application of solutions of 3-indoleacetic acid in lanolin. Arrows point to sites of application. Left: Solutions in U.S.P. lanolin. Right: Solutions in purified U.S.P. lanolin. Top: 0.01% solutions of 3-indoleacetic acid. Center: 0.001% solutions of 3-indoleacetic acid. Bottom: 0.0001% solutions of 3-indoleacetic acid.

and 0.0001% indole-3-acetic acid were prepared both in U.S.P. anhydrous lanolin and in U.S.P. anhydrous lanolin which had been treated with sodium hydrosulphite. Five to ten mg. of each lanolin solution were applied unilaterally to the first internode of each of six bean[2] seedlings 72 hours after

[2] Variety *Tendergreen*, Ferry Morse Seed Co. Stock No. H6020.

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emergence from the soil. Six hours after treatment three of the test plants from each group were photographed.

Distinct negative curvatures resulted from application of the 0.01% solutions of indole-3-acetic acid in both solvents (fig. 1). However, the growth substance in purified lanolin caused a more pronounced curvature than in non-treated lanolin. The application of a 0.001% solution in peroxide-free lanolin produced a marked response, while that in non-treated lanolin gave no perceptible curvature. A 0.0001% solution in either of the solvents produced no curvatures.

The use of U.S.P. anhydrous lanolin as a diluent for growth substances sensitive to peroxides may introduce an error of between 10- and 100-fold into quantitative assays. Caution must be observed in interpreting the results of assays in which the lanolin employed is assumed, without experimental justification, to be a completely inert solvent.

Summary

Lanolin has been found to contain sufficiently high concentrations of oxidizing agent(s) to interfere seriously with its use as a solvent for quantitative bio-assay of indole-3-acetic acid and for plant growth regulators in corn pollen. A method for removing the oxidizing agents from lanolin with sodium hydrosulphite is described.

This study was supported in part by the Rackham Research Endowment.

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NOTES

Executive Committee and Business Meetings, December 26 and 28, 1949.—

1. The report of the Secretary as of September 2 was approved. It was estimated that Society expenditures would exceed receipts of approximately $1,000.00 by July 1, 1950, if the present rate of printing is continued.

2. To minimize the probable deficit, it was voted to raise all classes of dues $1.00: that is, domestic dues will be $6.00; Canadian, $6.25; foreign, $6.50.

3. The following appointments were voted: Dr. W. F. Loehwing was reappointed to the Editorial Board to continue as Editor-in-Chief for a five-year term ending in 1954. Dr. F. P. Cullinan was appointed to the Editorial Board for a five-year term to expire in 1954.

4. Dr. A. G. Norman, Representative in Council of A.A.A.S., term to expire 1952.

5. Dr. H. H. Borthwick was appointed as our representative on the Editorial Board of the American Journal of Botany.

6. Two members of the Monograph Board were appointed. Dr. J. H. C. Smith was appointed to complete an unexpired term ending in 1950. Dr. Paul Kramer was appointed for the term ending in 1952.

7. The Executive Committee approved the proposal to change Article IX to read "that the Editorial Board of PLANT PHYSIOLOGY shall consist of 16 members instead of eight." This recommendation will be presented to the entire membership in 1950 for vote by mail ballot.

8. The business meeting included reports by the Treasurer and the Editor-in-Chief. Membership will probably reach 830 in the year ahead; with an anticipated mailing list, including institutional subscriptions, of approximately 1700 copies of the journal.

Dr. E. J. Kraus, Honorary Barnes Life Member.—This award is a token of the recognition of outstanding contributions in the field of Plant Physiology. It goes this year to a scientist who richly deserves this distinction, Mr. E. J. Kraus.

Plant and Soil Water Relationships.—PAUL J. KRAMER. McGraw-Hill Book Company, Inc., 330 West 42nd Street, New York 18, New York. 347 pages, 1949. $4.50.

This book presents the fundamental principles governing plant and soil water relationships and their applications to plant growth. It surveys an extremely wide range of investigative work including that of the author and correlates the subject matter in a highly functional manner, thus providing a good general understanding of the field. The book will be of considerable value to teachers, research workers and to students, all of whom will find the critical evaluations and conclusions of the author very helpful, especially in relation to those phases of the subject in which conflicting

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viewpoints exist. As the title implies, emphasis is given to edaphic factors rather than to climate in relation to plant processes and composition. Discussion of transpiration, ascent of sap and adaptations to drought are treated only incidentally because of their rather indirect relationship to water absorption.

The author first traces the historical development of current viewpoints on water relations of plants and then presents a description of the physicochemical aspects of soil water and the means of its measurement and control. The biological responses of the plant, such as root growth, sap pressures and exudation phenomena, water and solute absorption are correlated with the dynamic factors of the environment. The concluding section deals with absorption deficits and their effect on plants. The author has facilitated understanding by the general reader by avoidance of highly technical terminology without sacrifice of accuracy. The general format and fifty excellent text figures enhance comprehension of the material. The bibliography of nearly 700 complete and critically selected citations to original papers, arranged alphabetically by author, makes the book an invaluable reference work. The cross-referenced subject matter and author indices contribute materially to the book's usability.

Water in the Physiology of Plants.—A. S. Crafts, H. B. Currier, and C. R. Stocking. The Chronica Botanica Company, Waltham, Massachusetts; Stechert-Hafner, Incorporated, New York City. 240 pages. 56 figs. 49 tables. 1949. $6.00.

The authors present broad reviews on water in relation to the physiology of plants in the first portion of this monograph and in succeeding chapters consider specialized papers, thus adapting the book for class and reference use. Special attention has been given to terminology and to methods to facilitate understanding of newer and older terms and to correlate the work of biologists, chemists, and physicists, thereby making the book an important contribution to the physical sciences as well as to biology. Biological data are interpreted as far as possible in relation to recent physicochemical concepts of water. Convincing arguments are presented for a distinction between osmotic pressure and imbibition. This mode of exposition should facilitate considerably much cooperative and highly productive research in biology and related sciences.

The monograph contains the following major topics: Structure of Water; Properties of Solutions; Osmotic Pressure, Osmosis and its Mechanism; Water as a Plant Component and Intracellular Distribution of Water; Osmotic Quantities of Plant Cells; Active Cell Water Relations; Uptake and Movement of Water in Plants; Water Loss and Retention. The book concludes with nearly 700 citations to original literature and comprehensive author and subject indices.

Electron Microscopy Techniques and Applications.—RALPH W. G. WYCKOFF. Interscience Publishers, Inc., 215 Fourth Avenue, New York 3, New York. 248 pages, 1949. $5.00.

The first portion of the book deals with the construction and operation of electron microscopes and methods of preparation of materials for study. There follows a remarkably well illustrated survey of applications already made of such methods, consisting largely of data obtained directly by the author. The concluding section of the book deals with the importance of the electron microscope in relation to the visibility of macromolecular particles and the resulting experimental significance of this advance. The major sections of the book comprise: Electron microscopes and their adjustment; Preparations for electron microscopy, metal shadowing, and use of surface replicas; Electron microscopy of particle suspensions and viruses; Photography of macromolecules and the structure of macromolecular solids. There are 202 text illustrations, mostly electron photomicrographs, and a comprehensive bibliography arranged alphabetically by author for each chapter. Separate subject and micrograph indices conclude the book.

The format, organization, and clarity of text, the uniform excellence of illustrative material and critical evaluation of data make this book of value not only to scientists actively engaged in electron microscopy but to all those generally interested in the fine structure of organic and inorganic materials. Phenomenal as recent advances made by the electron microscope have already been, the data of this book make it evident that we are as yet merely on the threshold of greater progress in our knowledge of organisms and matter which will be made possible by this technique. The author makes it clear that considerable time will be required for interpretation of data already available and that applications of the techniques of electron microscopy to new fields, especially as the instrument itself is improved, are numerous and as yet rather unpredictable. In biology it is apparent that the electron microscope provides the means of enlarging the scope of cytology from the cellular to the molecular scale of organization, an advance which may easily prove to be as important as that made possible by the original perfection of the compound microscope.

Advances in Agronomy, Volume I.—A. G. NORMAN, Editor. Academic Press, Inc., 125 East 23rd Street, New York, New York. 439 pages, 1949. $7.50.

Advances in Agronomy provides an objective survey and critical review of agronomic research and practice. Various aspects of soil-crop relationships are discussed by specialists. The topics comprise: Plant growth on saline and alkali soils by Hayward and Wadleigh; New fertilizers and fertilizer practices by Jones and Rogers; Soybeans by Weiss; Clay minerals in soils by Gieseking; Alfalfa improvement by White; Soil microorganisms and plant roots by Clark; Weed control by Crafts and Harvey; Boron in

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soils and crops by Berger; Potato production by Ora Smith; and Fixation of soil phosphorus by Dean. Citations to original literature accompany each chapter. The format, composition and textual content of the book make it readily readable and very useful. The table of contents at the beginning of each chapter and the 23-page general author index further contribute to the usability of the book.

Elementary Plant Physiology.—STUART DUNN. Addison-Wesley Press, Inc., Cambridge 42, Massachusetts. 164 pages, 1949. $3.50.

This book is a brief text designed for a one semester course in elementary plant physiology. The chapters of the book are: Structure and function of the plant cell; Absorption; Transpiration and translocation; Mineral nutrition; Photosynthesis; Organic constituents of plants; Respiration; Growth and movement. Many of the notable advances of recent years in the knowledge of the physiology of plants are briefly mentioned. A separate unbound laboratory manual outlining 49 experiments with numerous references for each experiment is available for use with the text.

Selenium: Its Geological Occurrence and Its Biological Effects in Relation to Botany, Chemistry, Agriculture, Nutrition and Medicine.—S. F. TRELEASE and O. A. BEATH. Published by the authors. New York, New York. 292 pages, 1949. $5.50.

This book is the first comprehensive survey to be published on selenium and its significance in biology. It will have immediate practical value for many scientists in the western states as a reference volume with maps and geological tables showing the distribution of selenium in rocks and soils. Plant keys, drawings and photographs are included to aid in the identification of the plants which accumulate large amounts of selenium thus often being responsible for serious losses of livestock or serving as an index to selenium in the soil. Other sections on the accumulation of selenium by plants, selenium poisoning in animals, selenium in relation to public health, prevention and control of selenium poisoning, and the chemistry of selenium will be of general interest. A bibliography and author and subject indices are included.

Praktikum der Zell-und Gewebephysiologie Der Pflanze.—SIEGFRIED STRUGGER. Springer-Verlag, 20 Reichpietschufer St. (British Sector), Berlin W 35, Germany. 225 pages, 1949. 27.60 German Marks.

This is a second fully revised edition of this author's well known earlier book of 1936 as Volume II of the Pflanzenphysiologische Praktika series. New techniques, experiments and instrumentation on plant cell and tissue physiology are quite extensive. One hundred specific experiments and exact procedures are given in detail. The book comprises the following major sections: Techniques for preparation and microscopic examination of living cells and tissues; Introduction to cell morphology and experimental

cytology; Plasmolytic procedures and their applications; Permeability; Absorption and optical procedures for such study including vital staining methods; Translocation; Electro-cytology and histology. Comprehensive literature citations are given in connection with each chapter. A complete cross-referenced subject index facilitates rapid reference use of the book.

Frontiers in Colloid Chemistry.—R. E. BURK and OLIVER GRUMMITT, Editors. Interscience Publishers, Inc., 215 Fourth Avenue, New York 3, New York. 157 pages, 1950. $4.00.

This is the concluding volume of a series of publications resulting from the annual lectures, "Frontiers in Chemistry," at Western Reserve University. The book comprises the following sections each prepared by an expert and accompanied by a comprehensive bibliography: Role of adsorption in colloid science; Chromatographic adsorption analysis; Ion exchange with reference to synthetic resins; Sedimentation; Colloidal electrolytes, wetting agents and detergents; Organization of crystals and micelles of soap, solubilization and detergency. The various chapters are well organized and the book includes many original data and helpful figures.

Bananas.—HARRY W. VON LOESECKE. Interscience Publishers, Inc., 215 Fourth Avenue, New York 3, New York. 189 pages, 1949. $4.50.

This book is the first in a series on the chemistry, physiology and technology of economic crops under the editorship of Dr. Z. I. Kertesz. Critical and systematic treatment is given to soil, cultural conditions, climate, seasonal influences, variety, harvesting, storage, and handling as factors in variability of the chemical composition and biochemical behavior of the crop. The book also aims to bring the problems of food processors before production specialists in the interests of improved utilization of crops. The style and terminology employed permit specialists in one field to understand readily the contributions of those in another and the interrelations between them. The book comprises the following sections: History and growth of the banana trade; Structure and development including crop nutrition and morphology; Storage and ripening methods; Chemical changes during ripening; Transportation; Banana products; Nutritive value in health and disease; Banana diseases; and World banana production and trade. Each section carries numerous citations to original research and many helpful data from the literature are given in the 28 figures and 51 tables of the book. Agriculturists and plant physiologists will find this very concise publication of considerable interest.

Evergreen Orchards.—WILLIAM H. CHANDLER. Lea & Febiger, Washington Square, Philadelphia 6, Pennsylvania. 452 pages, 1950. $6.00.

This textbook deals with the structure and physiology of evergreen species, most of which are grown for their fruit as human food. It is written

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for those taking advanced courses but is also designed to provide undergraduate students with helpful information in the solution of difficult orchard problems and for the improvement of orchard practices. The tree and its fruit are taken up first, followed by a detailed discussion of the evergreen orchard environment. Full consideration is given of conditions favorable or unfavorable for production of fruit with good market qualities. Individual chapters are devoted to the characteristics of each of the more important species under consideration. Altogether, more than 80 species are given attention.

The first three chapters of the book present a general discussion in terms of fundamental principles of morphology, anatomy and physiology as these relate to the tree and its fruit, the evergreen orchard and its environment, the growing and training of evergreen orchard trees. The ensuing 16 chapters are devoted to culture of specific crops including citrus fruits and their relatives, avocado, papaya, tea, coffee, cacao, lichi, bananas, mango, cashew, olive, date, coconut, oil palms, nut trees and other species of tree fruits. The 687 citations in the bibliography have been carefully selected from among recent authoritative publications and they add immensely to the usefulness of the book. A very complete eight-page subject index facilitates rapid reference work.

Journal of Experimental Botany.—T. A. BENNET-CLARK, Editor. Oxford University Press, Amen House, Warwick Square, London, E.C. 4. 1950. 35 s. per year.

This new journal has been founded by the British Society for Experimental Biology to provide a medium for the publication of original research in plant physiology biochemistry, and biophysics, and in such related fields as experimental agronomy. The Editorial Committee consists of Professor E. Ashby, Professor T. A. Bennet-Clark, Professor G. E. Blackman, Dr. R. Brown, Professor F. G. Gregory, Dr. W. O. James, Professor W. H. Pearsall, Dr. R. D. Preston, and Professor M. Thomas. The journal will not be restricted to papers sumbitted by members of the Society. Contributions from research workers, both in Great Britain and abroad, will be welcomed. Manuscripts of papers submitted for publication should be addressed to Professor T. A. Bennet-Clark, University of London, King's College, Strand, London, W.C. 2. Three numbers will comprise a volume and they will appear at four-monthly intervals, commencing in January 1950. The subscription price for three consecutive numbers is 35s., post free. Single numbers are 14s. Application for membership in the Society, £2 per year including the journal subscription, should be addressed to Dr. J. W. L. Beament, Department of Zoology, Downing Street, Cambridge, England.

Metabolism and Function.—D. NACHMANSOHN, Editor. Elsevier Publishing Company, Inc., 215 Fourth Avenue, New York 3, New York. 348 pages, 1950. $7.00.

This collection of papers in honor of Dr. Otto Meyerhof deals with topics of general physiological importance of which the following will be of special interest to plant physiologists: Triose-phosphorsäure als Intermediärprodukt bei der Zuckergärung mit intakter Hefe by W. Kiessling; Configurational relationships between naturally occurring cyclic plant acids and glucose by G. Dangschat and H. O. L. Fischer; Synthese et utilisation de l'amidon chez un flagelle' sans chlorophylle incapable d'utiliser les sucres by A. Lwoff, H. Ionesco, and A. Gutmann; Quantum efficiency of photosynthesis by O. Warburg, D. Burk, V. Schocken, and S. B. Hendricks.

The Use of Auxins in the Rooting of Woody Cuttings.—K. V. THIMANN and J. BEHNKE-ROGERS. Harvard Forest, Petersham, Massachusetts. 344 pages, 1950. $1.00.

In this publication an attempt has been made to assemble all data of a quantitative or semi-quantitative nature on the effects of growth regulators on the rooting of cuttings of trees and shrubs. The references and critical data are clearly and concisely presented in tables which enable the rapid determination of the information which is available for any particular species or variety. Separate tables give the effects of wounding, nutrients, and vitamins on the rooting of cuttings. The small cost and great usefulness of this volume should make it a standard reference for all who are engaged in the vegetative propagation of plants.

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Any one actively interested in Plant Physiology is eligible for membership in the Society. Candidates for membership must be recommended by two members, and approved by executive committee, or by the vote of the Society at some annual or special meeting.

The payment of the annual dues to the Society entitles the members to receive the official journal, Plant Physiology. They will receive in addition the regular bulletins and announcements sent out periodically by the secretary. Members are invited to take a personal interest in the growth and development of the Society.

Manuscripts for Plant Physiology should be addressed to Prof. Walter F. Loomis, Department of Botany, State University of Iowa, Iowa City, Iowa. Drawings and photographs should not exceed 8.5 × 11 inches in size, should be numbered correctly on the back, and separate typewritten legends included with the text of the manuscript. Tables and cuts should be used sparingly, and the text presented in concise form. Instructions to authors for preparation of manuscripts are given in the January number, Volume 25, 1950, of Plant Physiology, pages 190–191.

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